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TABLE OF CONTENTS

KERR, WARWICK E., Genetic determination of castes in the genus Melipona . 143

MORGAN, DELBERT THOMAS, JR., A cytogenetic study of inversions in *Zea mays* 153

BISHOP, CHARLES J., Differential X-ray sensitivity of Tradescantia chromosomes during the mitotic cycle 175

HINTON, TAYLOR, A correlation of phenotypic changes and chromosomal rearrangements at the two ends of an inversion. 188

LINCOLN, RALPH E., and JOHN W. PORTER, Inheritance of *beta*-carotene in tomatoes. 206

DEMPSTER, EVERETT R., and I. MICHAEL LERNER, Heritability of threshold characters 212

WAGNER, R. P., C. H. HADDOX, R. FUERST and W. S. STONE, The effect of irradiated medium, cyanide and peroxide, on the mutation rate in Neurospora . 237

NOVITSKI, E., The transfer of mutant genes from small inversions 249

GECKLER, ROBERT P., Genetic changes induced by exposure to nitrogen mustard and their inheritance in *Paramecium aurelia*, Variety 4 253

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GENETIC DETERMINATION OF CASTES IN THE GENUS MELIPONA

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DIFFERENT mechanisms of caste determination are encountered in different genera of stingless bees (KERR 1946, 1947, 1948). In Trigona and in Lestrimelitta a phenotypic caste determination, long known in Apis, is observed: a larva develops into a queen or into a worker depending upon the food it receives. In Melipona, on the other hand, caste determination is genotypic. Fertile females (queens) are heterozygous in some species for two, and in other species for three, pairs of genes, homozygosis for any one of which makes the individual develop into a worker. A review of the previously published evidence, as well as new data, relevant to the problem of caste determination in Melipona are reported in the present article.

It should be noted that males of stingless bees, like those of other Hymenoptera, differ from females in having a haploid, instead of diploid, chromosome complement. Females of several species of Melipona are known to have 18, while drones have only 9 chromosomes. Spermatogenesis in Melipona follows the well-known Apis scheme. The first meiotic division is abortive, and results in expulsion of a cytoplasmic bud; the second division is equational, and a bud of cytoplasm containing a nucleus is produced which subsequently degenerates, while the remaining cell develops into a spermatid and a spermatozoon. Cytological abnormalities in spermatogenesis occur in Melipona, as they do in Apis, in testes of pupae exposed to temperature variations. The oogenesis in Melipona has not been studied owing to technical difficulties. The chromosome counts were made in cells of nerve tissue and in those of the ovarial wall.

RESULTS

Form and Position of Royal Cells in the Combs

In Apis, the queen cells are much larger than those of workers and drones, and are localized principally at the bottom of the comb. In Trigona, the difference in size between queen cells and worker cells varies from species to species. Royal cells are localized peripherally in the horizontal combs.

A statistical study was made of the distribution and localization of different cells in combs of various species of Melipona and a random distribution was found with regard to queen and worker cells, while the drone cells were found chiefly at the center. All cells are of the same size in all three castes of Melipona.

Morphological Differences of the Castes in Melipona

Queens: Virgin queens are smaller than the workers, with a narrower head, eyes and thorax. The abdomen swells considerably after fertilization. Body color, in most species, is darker in the queen than in the workers and drones, and without marks. The queens are somewhat more precocious in their development than the workers (data for *M. quadrifasciata*):

egg	4.5	to	6.5	days
larva	7	to	8	days
prepupa	4.5	to	5	days
pupa	11.5	to	15	days
Total	30	to	34	days

Virgin queens, after emerging in the presence of the mother queen, are killed by the workers, or die within 15 days. If no fecundated queen is present, then one, or under exceptional conditions more young queens, after their flight and fecundation, are given a special diet for 6–10 days in strong hives and for 20 to 30 days in weak colonies. During this time the sexual organs complete their development and the egg laying may begin.

Workers: Workers have undeveloped ovaries, but in rare instances they may lay unfertilized eggs which produce only drones. The morphological characters of this caste are mainly used by taxonomists for the identification of species.

Generally the workers have more brilliant colors than the queens: For instance, in *M. quadrifasciata*, *M. favosa*, *M. scutelaris*, *M. interrupta* and others, the queens are all brownish and the workers are black banded with yellow, brown banded with yellow, or black banded with white.

The development follows approximately the following time table (data for *M. quadrifasciata*):

egg	4.5	to	6.5	days
larva	7	to	8	days
prepupa	5	to	5.5	days
pupa	15.5	to	18	days
Total	34	to	37	days

Drones: The main differences between males and females are the forms of the antennae and the genitalia. The antenna has 13 segments in males and 12 in females.

Frequency and Determination of the Castes

Combs with a variable number of cells were taken and the caste of each bee was registered either in the pupal stage or a short time after emergence in the laboratory.

These counts show that the proportion of males (drones) in a colony is not fixed but varies in accordance with the age of the colony and with the season. At certain times most males are killed off by the workers; but in the period preceding the nuptial flight even males from other colonies are admitted and are permitted to move around in the hive without being molested.

In contrast to this, the proportion of queens and workers in colonies is constant, except under certain abnormal conditions to be discussed later. As shown in table 1, in *Melipona marginata* and subspecies a ratio of 3 workers:1 queen is observed. But in *Melipona quadrifasciata, Melipona schencki, Melipona fuscata melanoventer*,[1] *Melipona favosa orbignyi, Melipona interrupta fasciculata, Melipona flavipennis, Melipona rufiventris paraensis*,[1] the ratio is 7 workers:1 queen (tables 2–8). The occurrence of these ratios suggests at once the following hypothesis of caste determination.

TABLE 1

Melipona marginata.

(χ^2 computed on the basis of a 3:1 expectation for the ratio workers:queens)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
	1946					
5	3-24 to 4- 1	34	14	0.44	0	(1)
5	4- 1 to 4-14	83	27	0.01	0	(1)
5	6- 2 to 6-18	118	11	18.67	0	(3)
9	6- 8 to 6-18	73	2	19.95	0	(3)
9	7-23 to 8- 4	158	11	30.82	0	(3)
5	8-18 to 8-25	14	0	4.67	0	(3)
5	8-21 to 9- 3	9	1	1.20	45	(3)
9	9-18 to 9-24	62	12	3.05	0	(1)
15	11- 2 to 11-10	32	6	1.72	4	(1)
15	11- 2 to 11-10	39	7	2.34	34	(1)
15	11-13 to 11-18	1	0	—	17	(1)
15	12-28, 1946 to 1-11, 1947	60	21	0.04	3	(1)
	1948					
22	1- 1 to 1- 2	20	1	4.59	5	(2)
22	1- 4 to 1- 6	89	2	25.23	0	(2)
76	4- 1 to 4- 5	27	7	0.35	0	(1)
Total	(1)	338	94	2.42		
Total χ^2 (1); nf=7				7.96		

* (1)—strong hives; (2)—weak hives; (3)—winter.

Since Melipona males are haploid, all gametes which they produce are, barring mutation, genotypically alike. Females are, on the contrary, diploid, and may accordingly produce several kinds of gametes. We assume, then, that the queens of *Melipona marginata* are always heterozygous for two pairs of genes, *AaBb*, homozygosis for any one of which makes a diploid individual a worker. Males would, then, be of four kinds, any one of which would produce, when mated to a queen, a ratio of 1 queen:3 workers among the female offspring, as follows:

[1] These names are given in accordance with the new classification of the subspecies of *M. fasciata*, by MOURE and KERR (1949).

Queen × Drone = 1 Queen + 3 workers

$AaBb \times AB = AaBb + (AABB + AABb + AaBB)$
$AaBb \times Ab = AaBb + (AABb + AAbb + Aabb)$
$AaBb \times aB = AaBb + (AaBB + aaBB + aaBb)$
$AaBb \times ab = AaBb + (Aabb + aaBb + aabb)$

For other species of Melipona, in which the ratio 1 queen: 7 workers has been found, we assume that a queen is always heterozygous for three pairs of genes, *AaBbCc*, homozygosis for any one of which makes a diploid individual a worker. The possible types of matings are, then, as follows:

Queen × Drone = 1 Queen + 7 workers

$AaBbCc \times ABC = AaBbCc + (AABBCC + AABBCc + AABbCC + AABbCc + AaBBCC + AaBBCc + AaBbCC)$
$AaBbCc \times ABc = AaBbCc + (AABBCc + AABBcc + AABbCc + AABbcc + AaBBCc + AaBBcc + AaBbcc)$
$AaBbCc \times AbC = AaBbCc + (AABbCC + AABbCc + AAbbCC + AAbbCc + AaBbCC + AabbCC + AabbCc)$
$AaBbCc \times Abc = AaBbCc + (AABbCc + AABbcc + AAbbCc + AAbbcc + AaBbcc + AabbCc + Aabbcc)$
$AaBbCc \times aBC = AaBbCc + (AaBBCC + AaBBCc + AaBbCC + aaBBCC + aaBBCc + aaBbCC + aaBbCc)$
$AaBbCc \times aBc = AaBbCc + (AaBBCc + AaBBcc + AaBbcc + aaBBCc + aaBBcc + aaBbCc + aaBbcc)$
$AaBbCc \times abC = AaBbCc + (AaBbCC + AabbCC + AabbCc + aaBbCC + aaBbCc + aabbCC + aabbCc)$
$AaBbCc \times abc = AaBbCc + (AaBbcc + AabbCc + Aabbcc + aaBbCc + aaBbcc + aabbCc + aabbcc)$

Data

The actual data to which these hypotheses are to be applied are, according to species, as follows:

a) *Melipona marginata* (table 1)—We studied first the 3:1 type, found only in *M. marginata* and its subspecies. The colonies studied were collected in the States of Santa Catarina, São Paulo and Minas Gerais, in Brazil.

b) *Melipona quadrifasciata* (table 2) with subspecies: *quadrifasciata*, living in southern Brazil (southern part of São Paulo State, and the States of Paraná, Santa Catarina, and Rio Grande do Sul) and adjacent countries (Argentina and Paraguay); and *anthidioides*, living in the northern part of São Paulo State and all States to the north as far as Pernambuco. This species has been studied in most detail owing to its high frequency in our region. The colony number 4 is hybrid of the subspecies *quadrifasciata* × *anthidioides*.

c) *Melipona schencki*, (table 3) was collected in the State of São Paulo. Its domestication is harder than that of the former species.

d) *Melipona fuscata* subspecies *melanoventer* (table 4)—This subspecies is found only in the lower Amazon region, and colony 90 was examined by us during our trip to that zone.

e) *Melipona favosa* (subspecies *orbignyi*) (table 5)—Only one colony from State of Mato Grosso between the basin of Paraguay and Paraná rivers.

f) *Melipona interrupta* (subspecies *fasciculata*) (table 6) collected in the State of Maranhão.

TABLE 2

Melipona quadrifasciata.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
	1946					
1	2-16 to 2-27	69	8	0.31	0	(1)
1	2-20 to 3- 5	144	18	0.29	0	(1)
2	2-27 to 3- 7	52	8	0.04	0	(1)
3	3- 6 to 3-17	45	10	1.67	0	(1)
4	3-14 to 3-26	56	4	1.87	0	(1)
4	3-26 to 3-28	29	4	—	0	(1)
3	5- 9 to 5-16	52	2	3.82	0	(6)
2	5-14 to 5-24	73	2	6.63	0	(6)
3	5-28 to 6- 6	56	2	4.34	0	(6)
8	6-20 to 7- 1	43	2	2.67	0	(6)
1	8- 9 to 8-16	79	3	5.86	1	(6)
8	8-12 to 8-22	77	4	4.23	57	(6)
12	8-12 to 8-17	31	2	—	0	(6)
3	9-13 to 9-17	85	1	10.64	0	(6)
1	9-16 to 9-22	40	0	5.71	0	(6)
2	9-29 to 10- 6	78	8	0.80	2	(1)
1	10-16 to 10-24	111	4	8.56	0	(2)
14	10-26 to 11-10	57	9	0.08	15	(1)
2	11-11 to 11-19	91	8	1.14	0	(1)
1	11-28 to 12- 2	57	3	3.09	0	(2)
12	12-13 to 12-23	156	23	0.02	0	(1)
14	12-15 to 12-28	121	9	3.70	4	(3)
	1947					
12	1-17 to 1-25	97	12	0.22	7	(1)
12	2-19 to 2-28	83	2	8.22	0	(4)
12	2-22 to 3-11	177	17	2.48	106	(1)
12	4-10 to 4-23	100	20	1.90	28	(1)
12	5-24 to 6- 4	104	5	6.24	0	(6)
23	7- 1 to 7- 3	34	4	0.14	0	(5 and 6)
12	8- 4 to 8-23	93	9	1.26	0	(5 and 6)
23	9-20 to 9-21	25	1	—	0	(6)
35	11-10 to 11-25	61	13	1.74	20	(1)
	1948					
35	3- 1 to 3- 3	72	14	1.12	0	(1)
12	3-10 to 3-10	43	1	4.21	0	(1)
12	3-20 to 3-22	41	5	1.12	0	(1)
23	3-30 to 4- 7	57	2	4.48	0	(3)
8	4-10 to 4-14	83	9	0.62	0	(1)
35	5- 4 to 5- 8	54	10	0.57	0	(1)
35	5- 9 to 5-19	53	0	7.57	0	(6)
Total (1) and (5)		1632	214	1.39		
Total χ^2 of (1) and (5); nf=20				21.60		

* (1)—strong hives; (2)—parasitized by phorides (Dipt.); (3)—weak colonies; (4)—experimental weak colonies; (5)—colonies with temperature control and artificially fed; (6)—winter.

TABLE 3

Melipona schencki.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
	1946					
13	9-20 to 9-28	11	4	—	75	(1)
13	10-28 to 11- 2	71	8	0.41	20	(1)
13	11- 6 to 11-13	41	2	2.42	5	(1)
	1947					
13	1-26 to 2- 1	119	5	8.13	0	(3)
13	2-25 to 3- 2	147	9	6.46	2	(3)
13	3- 6 to 3-10	80	3	5.99	0	(3)
13	3-19 to 3-26	174	9	9.62	1	(2)
Total (1)		123	14	0.65		
Total χ^2 (1); nf=2				2.83		

* (1)—strong hives; (2)—experimental weak colony; (3)—bacterial infection.

g) *Melipona flavipennis* (table 7) was collected in the State of Pará (Amazon basin) but it occurs throughout northern and northeastern South America.

h) *Melipona rufiventris paraensis* (table 8) is a species living in the north of Brazil. Strong colonies have been found on the frontier of French Guiana, but attempts to maintain hives of this species in Piracicaba were unsuccessful, owing to its complete inadaptability to this climate.

TABLE 4

M. fuscata melanoventer.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
	1946					
16	11-18 to 11-20	28	2	—	0	(3)
	1948					
90	9-23 to 10- 2	90	7	2.48	30	(1) and (2)
90	10- 1 to 10- 7	106	9	2.30	83	(1)
90	10- 7 to 10-11	111	15	0.04	43	(1)
Total (1)		307	31	3.42		
Total χ^2 (1); nf=3				4.81		

* (1)—strong hive; (2)—In the Amazon valley in the period between December 15th and July 15th the rains are strong enough to interfere with the normal work of the bees. But in the "dry" season it often rains so much as to interfere somewhat with their work; (3)—weak hive.

TABLE 5

Melipona favosa orbignyi.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF LAYING OF EGGS	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
40	5-23 to 6-1, 1948	105	4	7.77	0	(2)
40	9- 1 to 9-5, 1948	49	8	0.12	0	(1)

* (1)—strong hive; (2)winter.

TABLE 6

Melipona interrupta fasciculata.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
82	11-29 to 12- 8, 1948	55	4	1.77	0	(1)
82	12- 8 to 12-13, 1948	27	7	2.03	0	(1)
89	3- 3 to 3- 9, 1949	44	9	0.97	0	(1)
Total (1)		126	20	0.19		
Total χ^2 (1); nf=3				4.77		

* (1)—strong hive.

TABLE 7

Melipona flavipennis.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
79-A	9-12 to 9-21, 1948	110	7	4.54	35	(1)
79-A	9-22 to 9-27, 1948	38	3	1.01	3	(1)
Total (1)		148	10	5.50		
Total χ^2 (1); nf=2				5.55		

* (1)—strong hive (see note 2, table 4).

TABLE 8

Melipona rufiventris paraensis.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
81	10-15 to 10-20, 1948	51	3	2.38	0	(1)

* (1)—strong hive.

DISCUSSION AND CONCLUSIONS

Table 1 contains data for 15 samples taken in 5 colonies of *Melipona marginata*. Among these, 8 samples (marked (1) in the column "Notes") were collected while the colonies were in good health and weather conditions were favorable; seven of these eight samples are large enough to be analyzed statistically. The χ^2 tests have been made taking 25 percent queens and 75 percent workers as the expected ratio (1:3 segregation).

Tables 2–8 contain data for 57 samples taken in colonies of seven species of Melipona. Among these, 34 samples (marked (1) or (5) in the column "Notes") were collected while the respective colonies were in good health and while weather conditions were favorable; 32 of these 34 samples are large enough to be analyzed statistically. The χ^2 tests have been made taking 12.5 percent queens and 87.5 percent workers as the expected ratio (1:7 segregation). It can be seen that the ratios observed fit the expected one as well as could be expected. The remainder of the samples (marked (2,) (3,) (4) or (6) in the tables) have been taken either during unfavorable season (May to early September, i.e., winter) or in weak or parasitized colonies. In such samples the percentages of queens fall below the expected 25 percent in *Melipona marginata*, and below 12.5 percent in the other species.

The reduction of the percentage of queens during winter is due to the lowering of the temperature of the hives rather than to any general effect of winter conditions. This has been shown experimentally by raising artificially the temperature of the colonies in winter; in such colonies the proportion of queens returns to normal (colonies 12 and 23 in table 2). Colonies with too few nurse bees, or those attacked by parasites or diseases, give low frequencies of queens even if the honey and pollen storage is sufficient. It can be shown experimentally that the loss of queens is also produced by deficient nourishment of the queen.

Several hypotheses that might account for these deviations have been considered in previous publications (KERR 1948):

a) Elimination of queens by the workers during the larval or pupal stage. This does not occur, since all cells are sealed after egg laying and are not opened again before emergence of the imago.

b) Preferential oösorption of the eggs which should produce queens. This hypothesis was rejected since there is not sufficient time after fecundation for a preferential degeneration of eggs.

c) Loss of chromosomes. Cytological aberrations were observed in two male pupae after they had been kept from 3 to 5 days at a low temperature (20°C) and it was supposed that the same could occur in the fertile female and, preferentially a female with one chromosome carrying a caste determinative gene. This cytological hypothesis was rejected because on counting the chromosomes of nearly 50 larvae during the winter it was found that all of them had 18 chromosomes.

d) From a poorly nourished egg (which has suffered little oösorption) a queen develops into a worker. This hypothesis is plausible but suggests that one could find intermediates; these were not found to date.

e) Abnormalities during meiosis and fertilization. 1—Preferential fertilization. 2—Parthenogenetic development with preferential fusion of two haploid nuclei or fusion of cleavage nuclei; if the egg be fertilized the male nucleus is eliminated. All these hypotheses are plausible, but at present the only hypothesis that fits the available data is that, under unfavorable conditions of temperature or of feeding, the male nucleus in fertilized eggs is absorbed or eliminated, the female pronucleus divides, and the division products fuse to give rise to diploid cleavage nuclei. Diploid females of such origin will evidently be homozygous for the caste-determining genes, and will therefore develop into workers instead of into queens. Owing to the technical difficulties of cytological investigation of bee eggs, no direct proof of this hypothesis has thus far been secured. The hypothesis is made plausible, however, by the fact that cases of female parthenogenesis have been reported in Hymenoptera by several authors. HASKINS and ENZMANN (1945) described such a process in ants, MACKENSEN (1943) in the honey bee, SMITH (1941) in Dyprion, etc. The effect of low temperature causing fusion of nuclei in eggs of Triturus has been observed by FRANKHAUSER (1942). Recently a promising new method for solution of this problem was found. Some genes affecting body color have been found in colonies of *Melipona marginata*. By following the segregations of these genes at different seasons, it should be possible to adduce conclusive evidence for or against diploid parthenogensis as the cause of the reduction of normal proportions of queens in colonies of this species. Thus far the data are small but they are consistent with the hypothesis. This topic will be discussed elsewhere.

SUMMARY

The development of a diploid larva into a queen or into a worker bee depends in several species of Melipona upon the genetic constitution of that larva. In *Melipona marginata* the queens are always heterozygous for two pairs of genes, *AaBb*. Homozygosis for either of these genes produces a worker. Males are either *AB*, or *Ab*, or *aB*, or *ab*. The mating of any of these males with the *AaBb* females gives segregation in a ratio of 1 queen: 3 workers among the diploid zygotes. In *Melipona quadrifasciata*, *Melipona schencki*, *Melipona fuscata melanoventer*, *M. favosa orbignyi*, *Melipona interrupta fasciculata*, *Melipona flavipennis* and *Melipona rufiventris paraensis* females are heterozygous for three pairs of genes (*AaBbCc*), homozygosis for any one of which makes a diploid zygote develop into a worker. In these species the normal ratios of

queens and workers in colonies approach 1:7. Deviations from these ratios are, however, observed in winter and in colonies that are attacked by parasites or diseases. These deviations may be accounted for by supposing that under certain conditions the male pronucleus is eliminated from the egg, and the division products of the female pronucleus fuse to give rise to diploid cleavage nuclei.

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LITERATURE CITED

FANKHAUSER, GERHARD, 1942 Induction of polyploidy in animals by extremes of temperature. Biol. Symp. **6**: 21–35.

HASKINS, CARYL P., and ERNST V. ENZMANN, 1945 On the occurrence of impaternate females in the Formicidae. J. N. Y. ent. Soc. **53**(4). 263–277.

KERR, W. E., 1946 Formação das castas no gênero Melipona (Illiger, 1806). An. Esc. Sup. Agr. "Luiz de Queiroz" **3**: 299–312.

1947 Estudos sôbre o gênero Melipona. Agr.D. thesis. Escola Superior de Agricultura "Luiz de Querioz," Universidade de S. Paulo.

1948 Estudos sôbre o gênero Melipona. An. Esc. Sup. Agr. "Luiz de Queiroz" **5**: 181–276.

MACKENSEN O.. 1943 The occurrence of parthenogenetic females in some strains of honey bees. J. Econ. Ent. **36**(3): 465–467.

MOURE, J., and W. E. KERR, 1949 Sugestões para a modificação da sistemática do gênero Melipona. Paper in the "II. Semana de Genética" in Piracicaba, Est. S. Paulo, Brazil.

SMITH, S. G., 1941 A new form of spruce sawfly identified by means of its cytology and parthenogenesis. Sci. Agric. **21**(5): 245-305.

A CYTOGENETIC STUDY OF INVERSIONS IN ZEA MAYS

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ALTHOUGH spontaneous and induced inversions have been reported in many organisms, the genetic behavior of inversions is known exclusively from studies of Drosophila. It is the purpose of this study to investigate inversions in *Zea mays*, at present the only organism other than Drosophila in which a relatively precise genetic and cytological analysis could be made.

MULLER (1940) proposed that inversions including the centromere be called pericentric and inversions limited to a single chromosome arm be designated as paracentric. Heterozygous inversions of both types form loop bivalents at meiotic pachytene or in the salivary gland chromosomes due to the pairing of the inverted and normally arranged homologues. Single crossover chromatids arising from exchanges within the limits of a heterozygous paracentric inversion are dicentric and form a chromatin bridge at meiotic anaphase or are acentric and lost because of their failure to undergo regular mitotic behavior (MCCLINTOCK 1933). The dicentric and acentric crossover chromatids carry genetic duplications and deficiencies. Single crossover chromatids arising from exchanges within heterozygous pericentric inversions carry genetic duplications and deficiencies but such chromatids each have a single centromere and no chromatin bridges are produced. Double crossover chromatids arising from multiple exchanges within either heterozygous pericentric or paracentric inversions do not carry duplications and deficiencies and may be recovered in viable offspring. Duplication-deficiency chromatids ordinarily cause abortion of the gametophyte generation of plants. In animals duplication-deficiency chromatids result in aneuploid gametes which, though functional, give rise to zygotic or embryonic lethals. Consequently, heterozygous inversions have been found to reduce greatly genetic recombination within the inversion segments in *Drosophila melanogaster* (STURTEVANT 1926; 1931; STURTEVANT and BEADLE 1936) and in *D. pseudoobscura* (DOBZHANSKY and EPLING 1948). In the inversion heterozygote, crossing over is also frequently reduced in the regions adjacent to the breakage points (STURTEVANT and BEADLE 1936; DOBZHANSKY and EPLING 1948).

Pericentric inversions are not abundant in natural populations, due apparently to the mortality caused by aneuploid gametes. Two spontaneous pericentric inversions have been reported in *Drosophila robusta* (CARSON and STALKER 1947) and one in *D. algonquin* (MILLER 1939). In contrast to the scarcity of spontaneous pericentric inversions, there is an abundance of para-

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centric inversions in natural populations of Drosophila. Unlike pericentric inversions, heterozygous paracentric inversions result in little or no mortality in Drosophila and Sciara. Dicentric duplication-deficiency crossover chromatids are excluded from functional egg nuclei, resulting in the production of eggs carrying non-crossover chromatids with full complements of genes (STURTEVANT and BEADLE 1936; CARSON 1946). Normal sperm are produced by Drosophila male inversion heterozygotes, since crossing over does not occur in the male. Meiotic anaphase bridges and fragments reported in many species of plants indicate that paracentric inversions are also frequent in plant populations. In general, crossing over occurs in both sexes in plants. Dicentric chromatids are excluded from the terminal megaspores in megasporogenesis (DARLINGTON and LA COUR 1941) but are included in non-functional, aneuploid microspores. Consequently, pollen abortion, but little or no ovule abortion results from heterozygous paracentric inversions in plants. However, plants produce an abundance of pollen, and the presence of a considerable proportion of aborted pollen presumably would have no great selective disadvantage. Therefore paracentric inversions might be expected to occur frequently in plant populations.

Although the cytological behavior of inversions in maize has been determined by MCCLINTOCK (1931, 1933, 1938), the genetic effects of heterozygous inversions have not previously been reported in plants. The present study comprises a cytological and genetic analysis of X-ray induced inversions 4a, 5a and 2b in maize and a cytological analysis of included inversion 2a/2b. For convenience in nomenclature the inversions studied are designated numerically according to the chromosome involved and alphabetically in the chronological order of their discovery.

MATERIALS AND METHODS

The cytological location of the two points of breakage in the inverted chromosome may be determined in the heterozygote from an analysis of changes of homology in the pachytene loop configuration. Microsporocytes were smeared in acetic or propionic carmine after aceto-alcohol fixation and the points of breakage determined by the use of a map measure from camera lucida drawings of pachytene configurations. If the two points of breakage in a pericentric inversion are not equidistant from the centromere, the inverted chromosome will have arm lengths different from its normally arranged homologue. Comparisons were made of pachytene arm lengths in normally arranged and homozygous inverted bivalents for inversions 5a and 2b.

To obtain the genetic location of the breakage points with reference to linked marker genes, backcrosses were made with the female parent heterozygous for the particular inversion and marker genes and employing the appropriate tester as the pollen parent. Inversion heterozygotes in a segregating backcross population in maize may be conveniently classified due to the pollen abortion resulting from crossing over. The two breakage points of an inversion, when cytologically established, may be used as physical landmarks in determining the cytogenetic position of genes situated on the same chromosome. Since sin-

gle crossover chromatids lead to abortion of the male and female gametophytes in inversion heterozygotes, all loci within the limits of the inversion are completely linked except for recombination effected by double crossover chromatids. Consequently, greatly reduced recombination values between two loci indicate that both may be within the inverted region or one within and one adjacent or both adjacent to the points of breakage and separated by the inversion. Recombination values between an inversion and loci outside the limits of the inversion may be determined by regarding the inversion as a dominant gene for pollen abortion and computing the amount of recombination between it and the marker gene.

The percentage of aborted pollen characteristic of the individual inversions was determined by staining and counting all of the pollen grains of each anther examined. Ovule abortion frequencies were determined from open pollinated ears for comparison with pollen abortion frequencies. The minimum genetic length of an inversion may be estimated from pollen abortion frequencies. The two breakage points of an inversion may be regarded as two loci separated by the length of the inversion with the products of recombination measured as aborted pollen. Each single or three strand double exchange within the inverted sector will produce two viable and two aborted pollen grains. Each four-strand double exchange will produce four aborted pollen grains. The percentage of pollen abortion will equal one-half of the single chiasma and three-strand double chiasma frequencies plus the four-strand double chiasma frequency. The percentage of pollen abortion is therefore a measure of the minimum genetic length of the inversion, since it represents a fairly accurate approximation of one-half the total chiasma frequency, with only double crossover chromatids from multiple exchanges remaining undetected, since they, like noncrossover chromatids, give rise to viable male gametophytes. Maize plants free from known chromosomal aberrations have varying amounts of aborted pollen, usually less than five percent. The percentage of aborted pollen in normal sister plants must be subtracted from the percentage of aborted pollen in the inversion heterozygotes in order to determine an approximation of the minimum genetic length of an inversion. Due to suppression of crossing over by asynapsis and non-homologous pairing (McCLINTOCK 1933), the genetic length of a heterozygous inversion would be less than the genetic length of the same sector in the inversion homozygote or in a normally arranged bivalent.

RESULTS AND DISCUSSION

Inversion 4a

X-ray induced paracentric inversion 4a was discovered and studied cytologically by McCLINTOCK (1938). She located the proximal break at a point approximately one-third of the length of the long arm from the centromere; the distal break was observed to be near the end of the long arm. A diagrammatic interpretation of inversion 4a follows (fig. 1).

Plate I, 1 confirms the observations of McCLINTOCK. Typical inversion loop configurations were not abundant at pachytene in heterozygous in-

version 4a plants, and analysis was often difficult due to frequent asynapsis and non-homologous pairing (plate I, 2).

In order to locate the position of inversion 4a on the genetic map of the fourth chromosome, plants heterozygous for the inversion were crossed with pollen from tester stock not carrying the inversion and homozygous for the recessive genes lazy (*la*), sugary (*su*) and glossy$_3$ (*gl*$_3$). ANDERSON and RANDOLPH (1945)

FIGURE 1. Inversion 4a. The centromere is indicated by the circle and the positions of the breakage points in the long arm by arrows. The heteropycnotic knob is located in the long arm.

place the centromere of chromosome 4 to the left of tunicate (*Tu*) in the long arm and nine units or more to the right of sugary in the short arm. A four-point test of *la su Tu gl*$_3$ by JENKINS (EMERSON, BEADLE, and FRASER 1935) placed *la* to the left of *su* and *gl*$_3$ to the right of *Tu*. The order of the marker genes employed in this study with respect to the centromere is thus *la su* centromere *gl*$_3$. A linkage map of chromosome 4 (fig. 2) has been assembled from data contained in EMERSON, BEADLE, and FRASER (1935) and ANDERSON and RANDOLPH (1945).

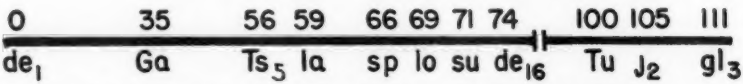

FIGURE 2. Linkage map of chromosome 4. In this and subsequent linkage maps, the approximate genetic position of the centromere is indicated by the cross lines.

F$_1$ plants heterozygous for the inversion and marker genes were backcrossed with pollen from homozygous tester plants with the normal gene order. Sister F$_1$ plants not carrying the inversion were backcrossed with *la su gl*$_3$ to obtain control values for crossing over. Table 1 presents control values for the *la—su* and *su—gl*$_3$ regions. Table 2 presents linkage data for the marker genes and the inversion from a backcross population of 751 individuals. The symbol *I* indicates presence of the inversion and *i* absence of the inversion. Classification for the inversion was effected by examining pollen from each plant. Inversion 4a is known to be located in the long arm of chromosome 4 with the proximal break one-third of the length of the long arm from the centromere. A study of table 2 shows a recombination value of 12.6 for *gl*$_3$ and the inversion. This indicates that the proximal break of the inversion is at least 13 units distal to the *gl*$_3$ locus in the long arm of chromosome 4. Consequently, the physical location of *gl*$_3$ is established within the proximal third of the long arm. The *la—su* interval is 7.5 units long in the inversion heterozygote stocks and 8.1 units long in control stocks. Crossing over in the short arm has not been affected appreciably by the inversion in the long arm. The *su—gl*$_3$ interval has been reduced from 28.0 in the control stock to 22.0 in the inversion heterozygote. The reduction in crossing over for the *su—gl*$_3$ interval in the heterozygous

TABLE 1

Genetic length of the la—su and su—gl_3 intervals.

Data from the cross $\frac{la\ su\ gl_3}{+\ +\ +} \times la\ su\ gl_3$.

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	*la su* gl_3	148
(0)	+ + +	172
(1)	*la* + +	20
(1)	+ *su* gl_3	14
(2)	*la su* +	57
(2)	+ + gl_3	75
(1–2)	*la* + gl_3	4
(1–2)	+ *su* +	2

Crossover value for *la—su* interval= 8.1% $\left(\frac{40}{492}\times 100\right)$

Crossover value for *su*—gl_3 interval=28.0% $\left(\frac{138}{492}\times 100\right)$

TABLE 2

Results from the cross $\frac{la\ su\ gl_3\ i}{+\ +\ +\ I} \times la\ su\ gl_3\ i$.

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	*la su* gl_3 *i*	210
(0)	+ + + *I*	260
(1)	*la* + + *I*	22
(1)	+ *su* gl_3 *i*	23
(2)	*la su* + *I*	76
(2)	+ + gl_3 *i*	59
(3)	*la su* gl_3 *I*	33
(3)	+ + + *i*	36
(1–2)	*la* + gl_3 *i*	3
(1–2)	+ *su* + *I*	3
(1–3)	*la* + + *i*	0
(1–3)	+ *su* gl_3 *I*	2
(2–3)	*la su* + *i*	5
(2–3)	+ + gl_3 *I*	16
(1–2–3)	*la* + gl_3 *I*	1
(1–2–3)	+ *su* + *i*	2

Crossover value for *la—su* interval= 7.5% $\left(\frac{56}{751}\times 100\right)$

Crossover value for *su*—gl_3 interval=22.0% $\left(\frac{165}{751}\times 100\right)$

Crossover value for gl_3—*I* interval=12.6% $\left(\frac{95}{751}\times 100\right)$

inversion stock as compared with the control is statistically significant at the five percent level. The suppression of crossing over is probably the result of reduced chiasma formation in the long arm between the centromere and the locus of gl_3 due to asynapsis and non-homologous pairing, which are often evident in the pachytene configurations of inversion 4a. Since none of the known genes in chromosome 4 lies within the inverted region, it was not possible to demonstrate that crossing over within the inversion is effectively suppressed.

Table 3 gives the pollen sterility of heterozygous inversion 4a plants. In table 4 the pollen sterility of sister plants not carrying the inverison is tabulated. The minimum genetic length of inversion 4a as determined from pollen abortion is 25.3 units (28.2 minus 2.9). Plants heterozygous for inversion 4a

TABLE 3

Single anther pollen counts from plants heterozygous for inversion 4a.

PLANT NO.	NORMAL POLLEN	ABORTED POLLEN	% ABORTED POLLEN
1	1185	470	28.4
2	2186	905	29.3
3	1793	638	26.2
4	1730	695	28.7

$$\text{Average pollen abortion} = 28.2\% \left(\frac{2708}{9602} \times 100\right)$$

TABLE 4

Single anther pollen counts from sister plants not carrying inversion 4a.

PLANT NO.	NORMAL POLLEN	ABORTED POLLEN	% ABORTED POLLEN
1	2303	68	2.9
2	2237	67	2.9

$$\text{Average pollen abortion} = 2.9\% \left(\frac{135}{4675} \times 100\right)$$

produce well-filled ears that are normal in appearance. Examination of two ears from plants heterozygous for inversion 4a disclosed an ovule abortion of 4.0 percent (25 aborted ovules; 595 viable ovules). The striking difference between pollen abortion (25 percent) and ovule abortion (4 percent) indicates either very unequal frequencies of crossing over within the inverted region in male and female flowers or the exclusion of single crossovers from functional egg nuclei. EYSTER (1922) found no significant difference in crossing over in male and female flowers for the *su—Tu* region of chromosome 4. Apparently in maize as in Drosophila dicentric single crossover chromatids carrying duplications and deficiencies are not ordinarily included in functional egg nuclei. A deficient chromatid from a second anaphase bridge due to a chiasma proximal

to the inversion and disparate to a chiasma within the inversion may occasionally enter a terminal megaspore. However, double exchanges of this type also produce two normal chromatids each with a single centromere. McCLINTOCK (1938) found 3.0 percent of the second meiotic anaphase configurations in microsporogenesis arising from such double exchanges in heterozygous inversion 4a plants. Presumably the majority of aborted ovules observed are due to four-strand double crossovers within the inverted region, resulting in duplication and deficiency in all four chromatids. McCLINTOCK (1938) found that 3.1 percent of the first meiotic anaphase configurations in microsporogenesis of inversion 4a heterozygotes had double bridges and double fragments due to four-strand double crossovers within the limits of the inversion. The close agreement between McCLINTOCK's observations and the percentage of aborted ovules indicates that female sterility of paracentric inversion heterozygotes in *Zea mays* largely results from four-strand exchanges within the limits of the inversion.

As shown previously in the linkage map of chromosome 4, the region distal to gl_3 in the long arm does not include any known mutant genes. The location of gl_3 within the proximal third of the long arm of chromosome 4 by the use of inversion 4a reveals that more than two-thirds of the long arm of chromosome 4 is devoid of known mutant genes. Measurement of chromosome 4 at pachytene shows the long arm to be 1.6 times as long as the short arm. Only three of the eleven accurately mapped loci of chromosome 4 are in the long arm, and all three are located within the proximal third of the long arm. Since the distal two-thirds of the long arm is devoid of known mutant genes, there is a disproportionate distribution of mutant genes in the two arms of chromosome 4.

Inversion 5a

Pericentric inversion 5a, discovered by McCLINTOCK (unpublished), involves one-half of the length of the long arm and an extremely short region adjacent to the centromere in the short arm of chromosome 5. The following diagram shows the location of the breakage points in the long and short arms.

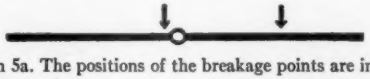

FIGURE 3. Inversion 5a. The positions of the breakage points are indicated by arrows.

An analysis of the pachytene bivalent involving the knobless inverted chromosome and its knobbed, normally arranged homologue shows the breakage point in the long arm to be near the large knob. Plate I, 3 shows the pachytene pairing of heterozygous inversion 5a. In figure 3 the two homologues are knobless. The long arm of chromosome 5 contains 52 percent and the short arm 48 percent of the total length of the pachytene chromosome (fig. 4). The ratio of the arms of the homozygous inversion 5a chromosome at pachytene is 3:1 (fig. 5). Due to the location of the breakage points, one-half of the chromatin in the long arm of normal 5 has been transferred to the short arm by the inversion, resulting in a rearranged chromosome with arm lengths strikingly different from the original.

FIGURE 4. Normal chromosome 5 bivalent at pachytene.

FIGURE 5. Homozygous inversion 5a bivalent at pachytene.

Note the change in arm ratios. The normal chromosome 5 is homozygous for a large knob in the distal half of the long arm. Camera lucida drawings ×900.

The following genetic map of chromosome 5 (fig. 6) was compiled by RHOADES (1940).

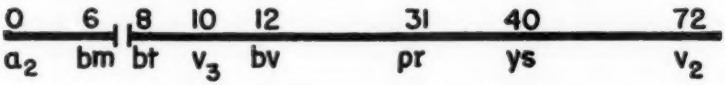

FIGURE 6. Linkage map of chromosome 5.

The position of the centromere on the genetic map of chromosome 5 was established by RHOADES (1936). Utilizing a fragment consisting of the centromere and the short arm of chromosome 5, RHOADES located the centromere between *bm* in the short arm and *bt* in the long arm. The *bm*—*bt* interval is only 2 map units long. Consequently, the centromere of chromosome 5 is marked on either side by loci with only 2 percent recombination.

To determine the position of inversion 5a on the genetic map of chromosome 5, plants homozygous for the inversion and *pr* were crossed with *a*$_2$ *bm bt Pr* pollen. F_1 plants were used as the female parent and backcrossed with *a*$_2$ *bm bt pr* pollen. RHOADES' study of the fragment of chromosome 5 placed the *a*$_2$ and *bm* loci in the short arm and the *bt* and *pr* loci in the long arm of chromosome 5. All F_1 plants were heterozygous for the inversion, eliminating the possibility of obtaining control linkage values from sister plants. However, the regions under consideration have previously been carefully studied, and extensive linkage data are available for comparison. Table 5 presents results obtained from crosses of

$$\frac{A_2\ Bm\ Bt\ pr\ I}{a_2\ bm\ bt\ Pr\ i} \times a_2\ bm\ bt\ pr\ i.$$

As may be anticipated from the length and location of the *bm*—*bt* interval and the proximity of the break in the short arm to the centromere, no crossing over

occurred between *bm* and *bt*. The *bm* and *bt* loci were completely linked by the inversion. For convenience the *bm* locus will not be recorded separately from the *bt* locus in subsequent discussions of the genetic behavior of inversion 5a. *Pr* and *pr* cannot be distinguished in homozygous a_2 seeds. In seeds homozygous or heterozygous for A_2, *Pr* produces purple aleurone and *pr* red aleurone. Seeds homozygous for a_2 have colorless aleurone. In computing the crossover value for the *bt*—*pr* region the A_2 class alone was utilized. In assembling the data of table 5, the reasonable assumption was made that class a_2 *bt pr* was numerically equal to its complementary crossover class A_2 *Bt Pr*. This assumption necessitated the removal of two individuals from the a_2 *bt Pr* non-crossover class. These corrections are recorded in parentheses. Since *Pr* and *pr* may not be distinguished in the a_2 classes, the presumed *Pr* and *pr* classifications for the a_2 classes are enclosed in parentheses. This assumption is justifiable since recombination in the *bt*—*pr* region is extremely limited (0.4 per cent) in the A_2 classes. A total of 848 mature plants, obtained from the 1090 seeds of table 5, were classified for the presence or absence of the inverted chromosome by determination of pollen abortion. The presence or absence of the inversion could not be accurately determined in 12 of the plants. In the remaining plants the inversion was found to be completely linked with the *Bt* locus. Recombination of the inversion with *Pr* was observed only in the two A_2 *Bt Pr* crossovers. The *Bt* locus may thus be conveniently and accurately used to follow the segregation of inversion 5a.

TABLE 5

Crossover values for the a_2-bt and bt-pr intervals in heterozygous inversion 5a plants.

$$\frac{A_2\ Bm\ Bt\ pr\ I}{a_2\ bm\ bt\ Pr\ i} \times a_2\ bm\ bt\ pr\ i.$$

GAMETIC TYPES	NO. OBSERVED
A_2 *Bt pr*	499
a_2 *bt* (*Pr*)	540 (538)
A_2 *bt Pr*	20
a_2 *Bt* (*pr*)	29
A_2 *Bt Pr*	2
a_2 *bt* (*pr*)	(2)
A_2 *bt pr*	0
a_2 *Bt* (*Pr*)	0

Crossover value for a_2—*bt* interval = $4.5\% \left(\frac{49}{1090} \times 100\right)$

Crossover value for *bt*—*pr* interval = $0.4\% \left(\frac{2}{521} \times 100\right)$

Table 6 gives additional data on recombination in the a_2—*bt* region in inversion 5a heterozygotes. Combining the data from tables 5 and 6 results in an average crossover value of 5.2 percent for the a_2—*bt* region.

TABLE 6

Data from the cross $\frac{A_2\ Bt\ I}{a_2\ bt\ i} \times a_2\ bt\ i$.

GAMETIC TYPES	NO. OBSERVED
$A_2\ Bt$	373
$a_2\ bt$	366
$A_2\ bt$	18
$a_2\ Bt$	31

Crossover value for the a_2—bt interval = 6.2% $\left(\frac{49}{788} \times 100\right)$

RHOADES (1941) made an intensive study of crossing over in the a_2—bt and bt—pr regions of chromosome 5. A significantly higher amount of crossing over was found in the male flowers as compared with the female. Moreover, high and low rates of crossing over for the a_2—bt region were observed in different lines. Normal and consistent rates of crossing over in male and female flowers were observed for the c—wx region of chromosome 9 in the same lines exhibiting unequal rates of crossing over for the a_2—bt region of chromosome 5. RHOADES concluded that the differences in crossover values observed for chromosome 5 were the result of some peculiarity inherent in that chromosome. Table 7 shows the different crossover values obtained by RHOADES for the $a_2 - bt$ region.

TABLE 7

Crossover values for $\frac{A_2\ Bt}{a_2\ bt} \times a_2\ bt$ *and the reciprocal cross (from* RHOADES *1941).*

	HIGH LINE, %	LOW LINE, %
Male flowers	26.3	13.4
Female flowers	17.3	5.9

A comparison of the crossover value of 5.2 percent for the a_2—bt region in the inversion heterozygotes with RHOADES' values of 17.3 and 5.9 for female flowers in the high and low lines indicates that a_2 is not located within the inversion in the short arm. Due to variation in crossing over in different lines for the a_2—bt region, it is not possible to determine definitely the degree of suppression of crossing over in the a_2—bt region in inversion 5a heterozygotes. RHOADES found a crossover value of 35.4 percent for the bt—pr region in male flowers and 30.3 percent for the same region in female flowers. The crossover value of 0.4 percent for the bt—pr region in the inversion heterozygotes indicates that the pr locus is within the limits of inversion 5a and that in maize as in Drosophila single crossover chromatids are not recovered from exchanges within a heterozygous inversion. Due to the inviability of single exchanges

within the inversion loop, the A_2 *Bt Pr* class necessarily arose from double exchanges in the *bt*—*pr* and *pr*—inversion break intervals within the inversion loop. The complete linkage of the inversion with *Bt* in the long arm shows the *bt* locus to be included within the inversion, as would be anticipated from RHOADES' study of the chromosome 5 fragment which originally located *bt* in the long arm. The absence of recovered crossovers between *bm* and *bt* in the progeny of the inversion heterozygotes shows that the *bm* locus is either included within the inversion or is closely adjacent to the break in the short arm.

Table 8 presents the results of pollen counts made from single anthers of plants heterozygous for the inversion and tester, and of plants homozygous

TABLE 8

Pollen abortion in inversion 5a heterozygotes and tester plants.

	VIABLE POLLEN	ABORTED POLLEN	% ABORTED POLLEN
Inv. 5a / 5 tester			
Plant 1	1454	877	37.6
Plant 2	1434	783	35.3
Plant 3	2009	1152	36.4
Plant 4	2109	917	30.3
5 tester / 5 tester			
Plant 1	1891	81	4.1
Plant 2	1990	151	7.1
Plant 3	1766	153	8.0

Average pollen abortion in heterozygous Inv 5a = 34.7% $\left(\frac{3,729}{10,735}\times 100\right)$

Average pollen abortion in homozygous 5 tester = 6.4% $\left(\frac{385}{6032}\times 100\right)$

for the tester chromosome. The amount of pollen abortion due to inversion 5a equals 34.7 percent minus 6.4 percent or 28.3 percent. The minimum genetic length of inversion 5a is 28 units.

A determination of ovule abortion frequencies revealed considerable disparity between the amount of ovule abortion and the amount of pollen abortion due to heterozygous inversion 5a. Seven ears from open-pollinated plants had a total of 315 aborted and 2192 viable ovules. The average ovule abortion of 12.5 percent was strikingly lower than the average pollen abortion of 28.3 percent. Since inversion 5a is a pericentric inversion, approximately equal amounts of ovule and pollen abortion would be expected. Apparently, in the inversion 5a heterozygote as in the normally arranged bivalent crossing over does not occur with the same frequency in male and female flowers.

Inasmuch as heterozygous inversions cause blocks of genes to be inherited

as a single unit, DOBZHANSKY and RHOADES (1938) suggested their use for a sector by sector analysis of the maize complement for genes of economic value. SPRAGUE (1941) utilized inversion 5a in an attempt to test for the existence of favorable genes located in the inverted segment. Stocks homozygous for inversion 5a and *pr* were crossed with an inbred line homozygous for *Pr*. The F_1 plants were backcrossed to the inversion stock. The resulting seeds were separated into classes homozygous for the inversion, *pr*/*pr*, and heterozygous for the inversion, *Pr*/*pr*. No significant differences were observed in the two classes for plant or ear height, number of ears, kernel row number or moisture content. The class heterozygous for the inversion, *Pr*/*pr*, showed increases, significant at the one percent level, in yield per plot and weight per 500 kernels due to favorable genes contributed by the inbred parent. The 12.5 percent ovule abortion characteristic of pericentric inversion 5a presumably caused a reduction in yield in the *Pr*/*pr* class. Even greater differences would undoubtedly have been observed if heterozygous inversion 5a had not produced ovule abortion. When possible, it would appear advisable to employ paracentric rather than pericentric inversions in chromosomal analyses for yield factors, since paracentric inversions, unlike pericentric ones, result in very little ovule abortion. However, pericentric inversions may be employed in an analysis for genes controlling plant characters not related to ovule abortion.

Inversion 2b

X-ray induced pericentric inversion 2b was discovered by RHOADES (unpublished). An analysis of pachytene configurations heterozygous and homozygous for the inversion showed that the inversion included the proximal .5 of the short arm and the proximal .15 of the long arm of chromosome 2. A diagrammatic interpretation of inversion 2b follows (Fig. 7).

FIGURE 7. Inversion 2b. The positions of the breakage points are indicated by arrows. The short arm is to the left of the centromere, which is indicated by the circle.

The unequal positions of the breaks in the long and short arms with respect to the centromere resulted in a rearranged chromosome with arms of very different lengths (fig. 8). In comparison with normal chromosome 2 (fig. 9) the inverted chromosome has gained chromatin in the long arm and lost chromatin in the short arm.

A study of heterozygous inversion 2b configurations revealed that the long arm of the inverted chromosome possessed a large knob located considerably nearer the centromere than the knob usually encountered in the long arm of chromosome 2. The break in the long arm was near but proximal to the knob (plate I, 4). Frequent non-homologous pairing was evident in bivalents heterozygous for inversion 2b. Many rod-shaped bivalents were observed in which the inverted sector was synapsed non-homologously. Plate I, 5 shows a

FIGURE 8. Pachytene bivalent of homozygous inversion 2b; knobbed. Camera lucida drawing ×900.

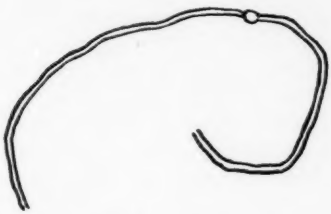

FIGURE 9. Pachytene bivalent of normal chromosome 2; knobless. Camera lucida drawing ×900.

heterozygous bivalent at pachytene exhibiting non-homologous pairing and asynapsis in the inverted and adjacent regions.

The genetic location of inversion 2b was determined by crosses involving the genes *ws*$_3$ *lg*$_1$ *gl*$_2$ *B* and *v*$_4$ in chromosome 2. The positions of these genes and of the centromere are shown on the following linkage map of chromosome 2 assembled from data in EMERSON, BEADLE and FRASER (1935), HAYES and IMMER (1942) and ANDERSON and RANDOLPH (1945). ANDERSON and RANDOLPH

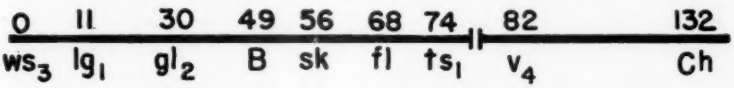

FIGURE 10. Linkage map of chromosome 2.

state that the map distance of the *ts*$_1$—*v*$_4$ interval is uncertain. They estimate the normal value to be approximately 20 units with the centromere five or more units from *ts*$_1$. Table 9 shows the linkage relationships of the inversion with *ws*$_3$*lg*$_1$ and *gl*$_2$. Table 10 presents data from sister plants not carrying the inversion, which were backcrossed with *ws*$_3$ *lg*$_1$ *gl*$_2$ pollen to obtain control values for the *ws*$_3$—*lg*$_1$ and *lg*$_1$—*gl*$_2$ intervals. The data from table 9 show that the break in the short arm of inversion 2b is 38 units proximal to the *gl*$_2$ locus. Thus *gl*$_2$ is physically located in the distal half of the short arm along with *ws*$_3$ and *lg*$_1$. According to the data in tables 9 and 10, crossing over is essentially normal in heterozygous inversion 2b plants for the *ws*$_3$—*lg*$_1$ and *lg*$_1$—*gl*$_2$ intervals. Table 11 shows the amount of recombination between *B* and the inversion break in the short arm. The gene order in the short arm is known to be *ws*$_3$ *lg*$_1$ *gl*$_2$ *B* with *B* located 19 units proximal to *gl*$_2$. The break in the short arm was located 38 units proximal to *gl*$_2$ by the data in table 9. Table 11 shows the break in the short arm to be 17 units proximal to *B*. Consequently *ws*$_3$ *lg*$_1$ *gl*$_2$ and *B* are physically located in the distal half of the short arm of chromosome 2.

According to the linkage map for chromosome 2, the total map length for

TABLE 9

Data from the cross $\frac{+\ +\ +\ I}{ws_3\ lg_1\ gl_2\ i} \times ws_3\ lg_1\ gl_2\ i.$

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	$+\ +\ +\ I$	147
(0)	$ws_3\ lg_1\ gl_2\ i$	139
(1)	$+\ lg_1\ gl_2\ i$	14
(1)	$ws_3\ +\ +\ I$	7
(2)	$+\ +\ gl_2\ i$	35
(2)	$ws_3\ lg_1\ +\ I$	32
(3)	$+\ +\ +\ i$	108
(3)	$ws_3\ lg_1\ gl_2\ I$	83
(1–2)	$+\ lg_1\ +\ I$	5
(1–2)	$ws_3\ +\ gl_2\ i$	0
(1–3)	$+\ lg_1\ gl_2\ I$	15
(1–3)	$ws_3\ +\ +\ i$	6
(2–3)	$+\ +\ gl_2\ I$	11
(2–3)	$ws_3\ lg_1\ +\ i$	8
(1–2–3)	$+\ lg_1\ +\ i$	1
(1–2–3)	$ws_3\ +\ gl_2\ I$	1

Crossover value for the ws_3—lg_1 interval= 8.0% $\left(\frac{49}{612} \times 100\right)$

Crossover value for the lg_1—gl_2 interval=15.2% $\left(\frac{93}{612} \times 100\right)$

Crossover value for the gl_2—I interval=38.1% $\left(\frac{233}{612} \times 100\right)$

TABLE 10

Data from the cross $\frac{+\ +\ +}{ws_3\ lg_1\ gl_2} \times ws_3\ lg_1\ gl_2.$

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	$+\ +\ +$	272
(0)	$ws_3\ lg_1\ gl_2$	210
(1)	$+\ lg_1\ gl_2$	27
(1)	$ws_3\ +\ +$	23
(2)	$+\ +\ gl_2$	46
(2)	$ws_3\ lg_1\ +$	50
(1–2)	$+\ lg_1\ +$	6
(1–2)	$ws_3\ +\ gl_2$	4

Crossover value for ws_3—lg_1 interval= 9.4% $\left(\frac{60}{638} \times 100\right)$

Crossover value for lg_1—gl_2 interval=16.6% $\left(\frac{106}{638} \times 100\right)$

the short arm has a minimum value of 79 crossover units. The 2b inversion break in the short arm was located cytologically in the center of the short arm and genetically 17 units to the right of *B*. Since crossing over may be reduced in regions adjacent to the inversion break, the length of the genetic map distal to the breakage point is 66 units as a minimum estimate. The genetic length of the entire short arm is approximately 79 units. That a disproportionately large amount of crossing over per unit of length occurs in the distal half of the short arm of chromosome 2 is clearly evident from the fact that the map length of the distal half is 66 units while the proximal half of the short arm has a map length of 13 units. McCLINTOCK (1931) located lg_1 within the very short region

TABLE 11

Recombination between the B locus and inversion 2b.

$\frac{B\ i}{b\ I}\times b\ i$		$\frac{b\ i}{B\ I}\times b\ i$	
GAMETIC TYPES	NO. OBSERVED	GAMETIC TYPES	NO. OBSERVED
B i	126	*b i*	49
b I	113	*B I*	48
B I	28	*b I*	9
b i	20	*B i*	13

Crossover value for the *B*—*I* interval = $17.2\% \left(\frac{70}{406}\times 100\right)$

consisting of the terminal four chromomeres of the short arm of chromosome 2. The locus of ws_3 is 11 units distal to lg_1. The interval between the locus of ws_3 and the end of the short arm is genetically unmarked. Consequently, there is a minimum chiasma frequency of 22 percent within the terminal four chromomeres of the short arm of chromosome 2. The surprisingly high chiasma frequency within the short sector composed of the terminal four chromomeres again illustrates the disproportionately high amount of crossing over per unit length in the distal region of the short arm of chromosome 2. Presumably, the lowering of exchange frequencies in the proximal region is controlled in some way by the centromere.

To determine the genetic location of the breakage point in the long arm of chromosome 2, plants heterozygous for the inversion and the marker genes gl_2 and v_4 were crossed with $gl_2\ v_4$ pollen. The data of table 9 disclosed that gl_2 was 38 units distal to the breakage point in the short arm. The locus of v_4 is 52 map units to the right of gl_2. RHOADES (EMERSON, BEADLE, and FRASER 1935) found 37 percent recombination in the gl_2—v_4 interval. ANDERSON and RANDOLPH found a minimum map distance of 7.3 units between the centromere and the locus of v_4 in the long arm. If the locus of v_4 were within the inversion they would show complete linkage except for double crossovers. Since inversion 2b is a comparatively short inversion, double crossovers would be expected to occur infrequently. The data of table 12 disclose 5.5 percent crossing over be-

tween v_4 and the inversion. Therefore, the locus of v_4 is 5.5 units distal to the inversion break in the long arm of chromosome 2. A crossover value of 38.1 per cent for the gl_2—I interval in the short arm was obtained from the data of table 9. The data of table 12 show a crossover value of 34.3 percent for the gl_2—I interval. The average crossover value for the gl_2—I interval computed from the data in tables 9 and 12 is 36.3 percent. The break in the short arm was located 17 units proximal to the B locus, and the break in the long arm 5 units proximal to v_4. Therefore, the region of chromosome 2 located 17 units proximal to B in the short arm and 5 units proximal to v_4 in the long arm is included

TABLE 12

Data from the cross $\frac{gl_2\ i\ v_4}{+\ I\ +} \times gl_2\ i\ v_4$.

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	$gl_2\ i\ v_4$	171
(0)	$+\ I\ +$	157
(1)	$gl_2\ I\ +$	79
(1)	$+\ i\ v_4$	92
(2)	$gl_2\ i\ +$	7
(2)	$+\ I\ v_4$	12
(1–2)	$gl_2\ I\ v_4$	6
(1–2)	$+\ i\ +$	4

Crossover value for the gl_2—I interval = 34.3% $\left(\frac{181}{528} \times 100\right)$

Crossover value for the I—v_4 interval = 5.5% $\left(\frac{29}{528} \times 100\right)$

within inversion 2b. This region corresponds to the proximal 0.5 of the short arm and the proximal 0.15 of the long arm of chromosome 2.

The recombination value for the gl_2—v_4 region in the inversion 2b heterozygotes is 36.0 percent. A comparison of the recombination value for gl_2—v_4 in the inversion 2b heterozygotes with the 37 percent recombination value obtained by RHOADES for gl_2—v_4 in normal stocks indicates no significant reduction of recombination due to inversion 2b. The lack of suppression of recombination in the gl_2—v_4 region is presumably due to the fact that the interval between the locus of gl_2 in the short arm and v_4 in the long arm includes a considerable portion of the length of chromosome 2, and that inversion 2b is a comparatively short inversion including regions adjacent to the centromere where recombination is less than in distal regions.

Table 13 shows the pollen abortion frequencies of heterozygous inversion 2b plants and sister plants not carrying the inverison. The average pollen abortion due to heterozygous inversion 2b was 19.1 percent while the ovule abortion was 20.1 percent.

TABLE 13

Pollen abortion frequencies of inversion 2b heterozygotes and normal sister plants.

	VIABLE POLLEN	ABORTED POLLEN	% ABORTED POLLEN
I/i (heterozygous inversion 2b)			
Plant 1	1456	502	25.6
Plant 2	2192	648	22.8
Plant 3	1739	524	23.2
Plant 4	2080	465	18.3
i/i (homozygous normal chromosome 2)			
Plant 1	2248	62	2.7
Plant 2	2035	57	2.7
Plant 3	2371	101	4.1

Average pollen abortion for $I/i = 22.3\% \left(\frac{2139}{9606} \times 100\right)$

Average pollen abortion for $i/i = 3.2\% \left(\frac{220}{6874} \times 100\right)$

Average pollen abortion due to Inv 2b = 19.1% (22.3% − 3.2%)

Cytological Analysis of an Included Inversion Heterozygote

Complex pairing configurations in the salivary gland chromosomes of Drosophila are found when two inversions involving the same chromosome are present (DOBZHANSKY and STURTEVANT 1938). According to the relative positions of the breakage points of the two inversions, complex inversion types are classified as included, overlapping or independent. Meiotic pairing of complex inversions has not been reported in any organism.

In order to study meiotic pairing of a complex inversion in *Zea mays*, plants heterozygous for two different inversions involving chromosome 2 were crossed. F_1 plants were examined cytologically for the presence of the complex inversion. Pericentric inversion 2b has previously been described in detail. Pericentric inversion 2a, discovered and studied by E. G. ANDERSON, includes 0.7 of the length of the short arm and 0.8 of the length of the long arm of chromosome 2. The location of the breakage points of inversion 2a produced a rearranged chromosome with arms less divergent in length than inversion 2 b (plate I, 4, and 6). The unequal location of the breakage points with respect to the centromere in inversion 2b, as previously described, resulted in a rearranged chromosome with strikingly different arm lengths.

Plate I, 4 and 6 show the pachytene configurations of heterozygous inversions 2a and 2b. Plate I, 7 shows the meiotic pairing of the inversion 2a/2b heterozygote. The abbreviated short arm and the large knob in the long arm of the inversion 2b chromosome enable it to be identified in the complex pachytene configuration. An analysis of figure 7 shows inversion 2b to be included

within inversion 2a. The naturally occurring included inversions analyzed cytologically by DOBZHANSKY and STURTEVANT in *Drosophila pseudoobscura* were of the paracentric type. The inversion 2a/2b configuration is an included pericentric type.

Pollen counts of individual anthers from two plants heterozygous for inversion 2a disclosed an average pollen abortion of 44.6 percent, which is considerably higher than the 19.1 percent abortion typical of inversion 2b. A plant free from any detectable aberration was crossed with pollen from a plant carrying inversion 2a/2b. Pollen samples were examined from 80 F_1 plants and classified into two groups for high and low pollen abortion. Of the F_1 plants 45 had the high pollen abortion characteristic of inversion 2a; 35 had the relatively lower pollen abortion characteristic of inversion 2b, indicating 1:1 segregation of inversion 2a and inversion 2b in microsporogenesis of the 2a/2b plant.

SUMMARY

A cytogenetic study was made of three inversions in *Zea mays*. One of these was a paracentric inversion with both breaks in the long arm of chromosome 4, while the other two involving chromosomes 2 and 5 were pericentric inversions.

Inversion 4a

As previously shown by MCCLINTOCK, the proximal break in the long arm is located at a point approximately one-third the length of the long arm from the centromere; the distal break is near the end of the long arm. Plants heterozygous for inversion 4a have an average pollen abortion of 25.3 percent and an average ovule abortion of 4.0 percent. The striking difference between pollen abortion and ovule abortion frequencies is attributed to exclusion of single crossover chromatids from functional megaspores, while single crossover chromatids are included in the microspores and cause their abortion. The proximal break of inversion 4a is located 12.6 units distal to gl_3, thus establishing the locus of gl_3 within the proximal third of the long arm of chromosome 4. The distal two-thirds of the long arm of chromosome 4 has no known mutant genes. The minimum genetic length of inversion 4a as determined from pollen abortion frequencies is 25.3 units.

Inversion 5a

The break in the short arm is located adjacent to the centromere; the break in the long arm is located at a point one-half the length of the long arm from the centromere. The break in the long arm is near the large knob in the long arm of chromosome 5. Plants heterozygous for inversion 5a have an average ovule abortion of 12.5 percent and an average pollen abortion of 28.3 percent.

The difference between the percentage of pollen and ovule abortion is attributed to higher frequencies of crossing over in male than in female flowers. The locus of a_2 is distal to the break in the short arm. The locus of *bm* is either proximal to the break in the short arm and included within the inversion or distal but very closely adjacent to the break in the short arm. The loci of *bt*

and *pr* are within the inverted region in the long arm. Single crossover chromatids are not recovered from exchanges within inversion 5a. Double crossover chromatids were recovered from exchanges within the *bt*—*pr* and *pr*—inversion break intervals in 0.4 percent of the eggs of the inversion heterozygotes. The *bt*—*pr* region is 23 units long in the genetic map of chromosome 5. Recombination within the *bt*—*pr* region of the inversion heterozygote is effected only by double crossover chromatids.

Inversion 2b

Inversion 2b includes the proximal .5 of the short arm and the proximal .15 of the long arm of chromosome 2. Plants heterozygous for inversion 2b show an average ovule abortion of 20.1 percent and an average pollen abortion of 19.1 percent. The break in the short arm is located 36.3 units proximal to gl_2 and 17.2 units proximal to *B*. The break in the long arm is 5.5 units proximal to the locus of v_4. Crossing over per unit length is considerably higher for the distal half of the short arm of chromosome 2 than for the proximal half.

Cytological Analysis of Included Inversion 2a/2b

The chromosome 2 bivalent of a plant heterozygous for the two pericentric inversions 2a and 2b was analyzed at pachytene. Inversion 2b was found to be included within inversion 2a. The 2a/2b plant showed a 1:1 segregation of inversions 2a and 2b in microsporogenesis.

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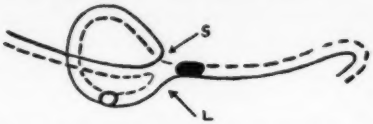
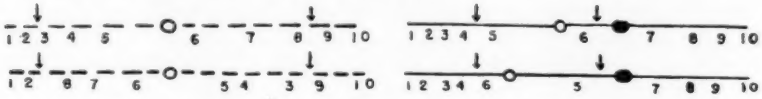
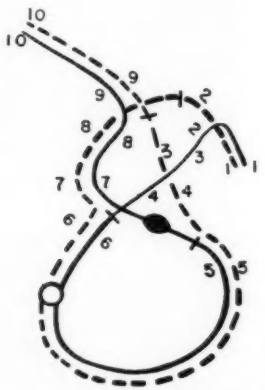


Diagram of plate I, 4. The inverted chromosome 2b is shown by a broken line. S represents the break in the short arm. L the break in the long arm.





Diagrammatic representation of plate I, 7. Inversion 2a is indicated by a broken line. Inversion 2b with the heteropycnotic knob in the long arm is indicated by a solid line. In the rod-shaped diagrams at the top of the figure the two inverted chromosomes are indicated, each below the normal chromosome 2 from which it was derived. The numerical sequence starts at the distal end of the short arm. The breakage points are indicated by arrows and the centromere by a circle.

Interpretation of the 2a/2b bivalent

The changes in homology and the approximate breakage points in 2a and 2b are indicated by the solid cross lines.

Region 1– 2 Normal arrangement distal to the inversion breaks in the short arms of 2a and 2b.

Region 3– 4 Inverted order for 2a; normal order in the short arm of 2b. Asynapsis is probably due to the much abbreviated short arm of inversion 2b.

Region 5– 6 Region inverted in common in 2a and 2b.

Region 7– 8 Inverted order for 2a; normal order in the long arm of 2b.

Region 9–10 Normal arrangement of 2a and 2b distal to the breakage points in the long arms.

Photomicrographs of pachytene configurations (×1800).

1. Heterozygous inversion 4a.

2. Heterozygous inversion 4a showing asynapsis and non-homologous pairing.

3. Heterozygous inversion 5a. One-half of the long arm is involved in the inversion loop. The centromere may be seen adjacent to the pycnotic region at the base of the loop.

4. Heterozygous inversion 2b. (See diagram)

5. Heterozygous inversion 2b showing asynapsis and non-homologous pairing in the inverted and adjacent regions.

6. Heterozygous inversion 2a.

7. The inversion 2a/2b bivalent. (See diagram)

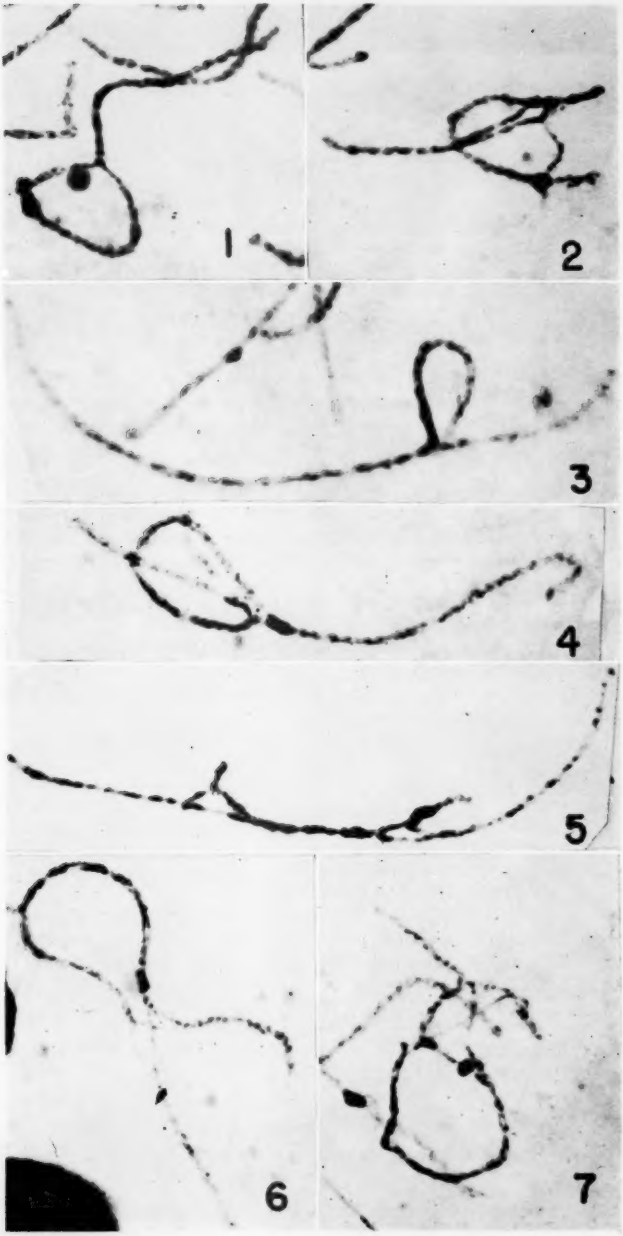


PLATE I

LITERATURE CITED

ANDERSON, E. G., and L. F. RANDOLPH, 1945 Location of the centromeres on the linkage maps of maize. Genetics **30:** 518–525.

CARSON, H. L., 1946 The selective elimination of inversion dicentric chromatids during meiosis in the eggs of *Sciara impatiens*. Genetics **31:** 95–113.

CARSON, H. L. and H. D. STALKER, 1947 Gene arrangements in natural populations of *Drosophila robusta* Sturtevant. Evolution **1:** 113–133.

DARLINGTON, C. D., and L. LA COUR, 1941 The genetics of embryo sac development. Ann. Bot. Lond. N. S. **5:** 547–562.

DOBZHANSKY, TH., and C. EPLING, 1948 The suppression of crossing over in inversion heterozygotes of *Drosophila pseudoobscura*. Proc. nat. Acad. Sci. **34:** 137–141.

DOBZHANSKY, TH., and M. M. RHOADES, 1938 A possible method for locating favorable genes in maize. J. Amer. Soc. Agron. **30:** 668–674.

DOBZHANSKY, TH., and A. H. STURTEVANT, 1938 Inversions in the chromosomes of *Drosophila pseudoobscura*. Genetics **23:** 28–64.

EMERSON, R. A., G. W. BEADLE and A. C. FRASER, 1935 A summary of linkage studies in maize. Cornell Univ. agric. Exp. Sta. Mem. **180:** 1–83.

EYSTER, W. H., 1922 The intensity of linkage between the factors for sugary endosperm and for tunicate ears and the relative frequency of their crossing over in microspore and megaspore development. Genetics **7:** 597–601.

HAYES, H. K., and F. R. IMMER, 1942 Methods of Plant Breeding. New York: McGraw Hill, xii+432 pp.

MCCLINTOCK, B., 1931 Cytological observation of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Univ. Mo. agric. Exp. Sta. Res. Bull. **163:** 1–48 pp.

1933 The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Z. Zellforsch. **19:** 191–237.

1938 The fusion of broken ends of sister half chromatids following chromatid breakage at meiotic anaphases. Univ. Mo. agric. Exp. Sta. Res. Bull. **290:** 1–48 pp.

MILLER, D. D., 1939 Structure and variation of the chromosomes in *Drosophila algonquin*. Genetics **24:** 699–708.

MULLER, H. J., 1940 Bearing of the 'Drosophila' work on systematics. In '*The New Systematics*.' J. S. Huxley, ed., Oxford: Clarendon Press, pp. 185–268.

RHOADES, M. M., 1936 A cytogenetic study of a chromosome fragment in maize. Genetics **21:** 491–502.

1941 Different rates of crossing over in male and female gametes of maize. Amer. Soc. Agron. **33:** 603–615.

SPRAGUE, G. F., 1941 The location of dominant favorable genes in maize by means of an inversion. Genetics **26:** 170.

STURTEVANT, A. H., 1926 A crossover reducer in *D. melanogaster* due to inversion of a section of the third chromosome. Biol. Zbl. **46:** 697–702.

1931 Known and probable inverted sectors of the autosomes of *D. melanogaster*. Carnegie Inst. Wash. Publ. 421, pp. 1–27.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X-chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21:** 554–604.

DIFFERENTIAL X-RAY SENSITIVITY OF TRADESCANTIA CHROMOSOMES DURING THE MITOTIC CYCLE

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MANY apparently conflicting results have been obtained by the various workers who have carried out experiments to determine the range of X-ray sensitivity of chromosomes at the different stages of the nuclear cycle. This sensitivity may be demonstrated through either structural or physiological effects on the cells.

By the observation of purely physiological changes, CARLSON (1942) working with living cultures of neuroblast cells of the grasshopper Chortophaga found mid to initial late prophase the most sensitive stage. KOLLER (1943) has shown that the physiological effects of X-rays on plant cells (microspores of Tradescantia) also indicate greatest sensitivity in the prophase.

On the basis of structural chromosome changes, each of the different stages has at some time and by some worker been considered the most sensitive. As cited by WHITING (1945a), earliest investigators in this subject, KRAUS and ZIEGLER (1906), and HOLTHUSEN (1921) agreed that the most sensitive stage of the nuclear cycle was at metaphase. MOTTRAM (1913) also came to the same conclusion. Later workers, however, have for the most part disagreed with these early findings, and until very recently the majority have considered some prophase stage to be the most sensitive and metaphase the least sensitive.

SAX and SWANSON (1941) found that the maximum sensitivity occurred just before mid-prophase and then decreased as metaphase was approached. CREIGHTON (1941) found the greatest sensitivity at the end of the resting stage or the very beginning of the prophase. DARLINGTON and LACOUR (1945) conclude from experiments on Trillium and Tradescantia that chromosomes in the condensed state are "either unbreakable by X-rays, or, if broken, always undergo prompt restitution."

Recent studies by WHITING (1940, 1945a, 1945b) based on the parthenogenetic development of X-rayed eggs of the wasp, Habrobracon, indicate that the metaphase stage is very sensitive. She has been able to correlate the gross results with cytological observations to some extent (WHITING 1945b), but the large number and small size of the chromosomes have limited the scope of this phase of the work. REYNOLDS (1941) investigated the effect of irradiation on the oöcytes of Sciara by observing the number of alterations in the salivary gland chromosomes of the resulting larvae. The effects were shown to be greatest when the oöcytes were X-rayed at the metaphase stage. BOZEMAN (1943) and BOZEMAN and METZ (1949) continued this work and found that sensitivity increased up to mid-anaphase and then decreased.

In most of these experiments, the results were based on survival of individuals or on chromosome alterations which were capable of being perpetuated over a period of many cell generations. Results from recent experiments using Trillium (SPARROW 1944, 1948) have been based on a cytological study of all the chromosome aberrations induced by the X-rays, including those lethal to cell reproduction. Though these observations were concerned with meiotic rather than mitotic divisions, they have confirmed the fact that metaphase is a point of high sensitivity. His work also shows that the apparent resistance of the metaphase stage found by some investigators and its high sensitivity found by others may be explained on the basis of "potential breaks" which are induced at stages when the chromosomes are in a condensed state, but which are not realized until after they pass through the diffuse state of the following resting stage.

In the present investigation, an attempt has been made to determine the stage and range of sensitivity in the mitotic divisions of pollen grains of Tradescantia, basing the observations on a direct analysis of chromosome alterations, and considering the fact that structural changes induced at one metaphase stage cannot be scored until the metaphase of the next nuclear division.

MATERIAL AND METHODS

The plants used were specimens of the diploid ($2N=12$) species, *Tradescantia paludosa* Anders. & Woodson, which were free of any naturally occurring centric fragments. Potted plants were used and were treated with X-rays by placing the pots on their sides and working the inflorescences into as narrow a vertical space as possible. X-raying was carried out at a distance of about 65 cm at an intensity of approximately 30r per minute.

The treatment dosage used was 250r, measured with an integrating-type dosimeter, the ionization chamber of which was placed in a median position among the inflorescences. It is estimated that the maximum range in the distance of the buds from the X-ray tube was 5 cm. At the distance used this meant a range of about 15 percent, or the dosage could be stated as 250r plus or minus 7.5 percent (18.75r). The X-ray machine was operated at a voltage level of 160 KV and 10 MA, and the radiation was unfiltered except for the inherent filtration of the X-ray tube. Exposures were made at room temperature of approximately 20°C.

Both before and after X-ray treatments the plants were kept in the greenhouse, remaining there until the buds to be used had opened. The pollen was then cultured in modified Van Tieghem cells using a 12 percent lactose-agar medium (BISHOP 1949). The X-ray effects were analyzed at the metaphase stage of the pollen tube division from slides prepared over a period extending from the second to the seventh day after treatment. All of the flowers used in the present experiment were obtained from a single plant. In most cases the pollen from any one flower was sown on more than one slide. These slides were scored separately, but since in no case was there any statistically significant difference between slides from the same flower, only the totals for each flower have been used.

RESULTS AND DISCUSSION

Nuclear changes.—Although the inflorescences of the plant were all treated with X-rays at the same time, the order of development of the buds in Tradescantia is such that each bud represents a different stage in the nuclear changes connected with the formation of the pollen grains. As only one bud in the series blossoms each day, the spacing of the stages is at about 24 hour intervals.

The sequence of cytological events is as follows: after the formation of the single-nucleate microspore at meiosis, there is about a 14 day period (the time varying somewhat with the season) before the pollen grain division. With this latter mitosis, the microspore becomes a binucleate pollen grain, and remains in that condition until the division of the generative nucleus which takes place after the pollen tube has almost reached its maximum development.

During the early part of this latter period, the chromosomes, following the despiralization process which takes place at late telophase, are single stranded, at least as far as X-ray effects are concerned. Thus, aberrations which are formed during this period result from the breakage of whole chromosomes. At a later stage the generative nucleus enters the prophase of the pollen-tube division. In Tradescantia this is initiated before the pollen is shed, and by a stage three days before anther dehiscence the chromosomes have become effectively double to X-rays. From this point on, X-rays may cause the breakage of only a single chromatid.

By an analysis of the aberrations found at the pollen tube metaphase, the two types (chromosome and chromatid breaks) may be readily distinguished, and from them may be obtained a direct indication of the nature of the chromosomes at the time of exposure to X-rays. Whole chromosome aberrations most commonly include small acentric fragments and dot deletions. More rarely, centric or acentric rings and chromosome exchanges resulting in dicentric chromosomes or simple translocations are also found. Chromatid aberrations, on the other hand, include chromatid exchanges, and at certain stages small chromatid deletions. When chromosome exchanges resulting in simple translocations are equal or near equal they are not detectable at the pollen tube metaphase, and therefore are omitted from the data presented. The frequency of these is probably relatively small, and would have little effect on the over-all picture.

In the present experiments, the period during which the chromosome sensitivity was studied extended from the sixth to the second day before anther dehiscence. This represented stages from just previous to the pollen grain mitosis up to the prophase of the generative nucleus division, and thus included both chromosome and chromatid aberrations.

Aberration types.—An examination of the frequency of the different types of aberrations (table 1) gives an indication of the variation in structure of the chromosomes of different buds at the time they were X-rayed. From this it is possible to correlate the changes in sensitivity with the changes in chromosome structure.

It will be noted in the table that up to the third day before dehiscence all the X-ray induced breaks involved whole chromosomes, but after that time

TABLE 1

Percentage types of aberrations produced by X-rays in Tradescantia. Dose 250r. Scored at pollen tube mitosis.

DAY*	FLOWER NO.	A.F.**	D.D.	R.	Dic.	Cs.E.	Ct.E.	TOTAL	N.
2	1	14.5	0.6	0.0	0.0	0.0	0.8	16.8	474
	2	14.2	0.8	0.0	0.0	0.0	0.4	15.8	240
	3	15.1	0.0	0.0	0.0	0.0	0.8	16.6	366
	Average	14.6	0.5	0.0	0.0	0.0	0.7	16.6	1080
3	1	34.6	5.3	0.3	2.2	0.0	0.3	45.6	318
	2	21.5	3.6	0.6	0.6	0.0	1.8	31.2	330
	Average	27.9	4.5	0.5	1.4	0.0	1.1	38.3	648
4	1	2.5	8.1	0.4	0.6	0.0	0.0	12.7	480
	2	14.0	7.9	0.3	0.3	0.0	0.0	23.3	618
	3	3.3	10.0	0.0	2.2	0.0	0.0	17.7	270
	Average	7.8	8.3	0.3	0.7	0.0	0.0	18.5	1368
5	1	21.8	20.6	0.2	2.6	0.0	0.0	48.0	504
	2	15.0	15.4	0.4	0.0	0.4	0.0	32.4	240
	3	3.3	9.0	0.6	1.4	0.2	0.0	16.6	846
	Average	10.9	13.7	0.4	1.6	0.1	0.0	28.9	1590
6	1	0.0	0.4	0.0	0.0	0.0	0.0	0.4	894
	2	0.0	0.2	0.0	0.0	0.0	0.0	0.2	600
	3	0.3	0.5	0.0	0.0	0.0	0.0	0.8	858
	Average	0.1	0.4	0.0	0.0	0.0	0.0	0.5	2352

* Days from X-raying to anther dehiscence.

** Abbreviations used:

A.F.—Acentric Fragment (Chromosome or isochromatid)
D.D.—Dot Deletion (Chromosome)
R.—Chromosome Ring
Dic.—Chromosome Dicentric
Cs.E.—Chromosome Exchange (Translocation)
Ct.E.—Chromatid Exchange
Total—Total % Aberration Frequency
N.—Number of Chromosomes

Note—The dot deletions on the second day were chromatid deletions. Percentages in all cases were calculated on the basis of the total number of chromosomes scored.

chromatid exchanges were also found. The latter can only be caused by the rupture of single chromatids, and indicate that the chromosomes first become effectively double on the third day. In those same slides, however, some chromosome dicentrics and rings were also present, showing that the process, although begun, had not yet been completed in all the cells. Those X-rayed on the second day before dehiscence contained chromatid exchanges and chromatid dot deletions, but no dicentrics or rings. On the basis of these results it would appear that the process by which the chromosomes become effectively

double to X-rays begins on the third day before dehiscence and is completed by the following day.

On this latter (second) day the majority of aberrations present were acentric fragments, either simple chromatid deletions or isochromatid breaks with sister chromatid reunion. Data collected by SWANSON (1940) show a majority of isochromatid breaks when the buds are X-rayed at the time that the flower opens, and a majority of single chromatid deletions when the pollen grains are X-rayed two hours after germination. These changes in aberration type are interpreted to indicate a progressive widening of the space between the two chromatids of each chromosome from the time of effective doubling two to three days before the pollen is shed, until by two hours after germination the strands are far enough apart that a single X-ray "hit" may break either strand independently.

Sensitivity Changes.—By an analysis of the frequencies of the different types of aberrations, it was possible to calculate the percentage of chromosome breaks induced at the different stages, and thus the comparative sensitivity of each. These data are summarized in table 2. From this table it may be seen that during the period studied there were two stages of high sensitivity, one at three days and the other at five days before the dehiscence of the anthers.

TABLE 2

Sensitivity of Tradescantia chromosomes at different times after X-raying with 250r. Scored at pollen tube metaphase.

TIME FROM X-RAYING TO DEHISCENCE	% BREAKS PER FLOWER			TOTAL BREAKS	TOTAL CHROM.	% BREAKS
	NO. 1*	NO. 2	NO. 3			
2 days	16.8	15.8	16.6	179	1080	16.6
3 days	45.6	31.2	—	248	648	38.3
4 days	12.7	23.3	17.7	253	1368	18.5
5 days	48.0	32.1	16.6	459	1590	28.9
6 days	0.4	0.2	0.8	12	2352	0.5
7 days	1.2	—	—	4	330	1.2

* Nos. 1, 2, and 3 represent different flowers used on any one day.

Practically no breaks were recorded at the pollen tube division for cells which were X-rayed on the sixth and seventh days before dehiscence. This is explained by the fact that the pollen grain mitosis occurs about five days before anther dehiscence, and any breaks induced before that time cause fragment loss at anaphase. These lost fragments form one to several micronuclei, within the pollen grain though outside the nucleus, their size depending on the number and type of fragments which form them. These were observed in slides of both the sixth and seventh days in considerable numbers. The pollen grains containing these micronuclei looked normal otherwise, but they either showed no signs at all of germination, of if they did, development proceeded for only a short time. In no case were any micronuclei observed in cells which had developed to the stage of the pollen tube metaphase.

Three-day Peak of Sensitivity.—If the chromosome sensitivity, as indicated in table 2 is considered in relation to the chromosome structure at the time of irradiation, it will be seen that one of the peaks of sensitivity is at the time when the chromosome is becoming effectively double to the action of X-rays. Closer examination of the two flowers from which pollen was germinated on that day (table 1) shows that flower No. 2 has the lower sensitivity. As it gave a higher number of chromatid exchanges and a lower number of rings and dicentrics combined than flower No. 1, it would seem that it was the older of the two. This is interpreted as evidence that the actual peak of sensitivity is at a point just before the chromosomes become effectively double to breakage by X-rays.

It may also be seen in both slides that there is a striking increase at that time in the number of acentric fragments that are not chromosome dot deletions. SAX (1941) has shown that acentric fragments produced after chromosome doubling represent almost entirely double chromatid deletions with sister chromatid reunion. Dot deletions, on the other hand, are considered by most investigators (RICK 1940; SAX and SWANSON 1941; NEWCOMBE 1942; KOLLER 1943; DARLINGTON and LACOUR 1945) to be minute rings, probably formed in many cases by the deletion of a single relic coil of a chromosome when in the early prophase stage. In general, dot deletions are formed before chromosome doubling, acentric fragments after it occurs.

Because of the observed prevalence of acentric fragments at the peak of sensitivity on the third day, it is suggested that at a point just before the chromosomes become effectively double to breakage by X-rays, but after they are actually double, there is a stage where a single X-ray "hit" will normally cause a rupture of both chromatids. Because of the presumably very close proximity at this stage, the chances of sister chromatid reunion are almost, if not as great as those of restitution. This, then, represents the point of high sensitivity. Later in the cycle, even though an X-ray might break both sister chromatids with a single ionization path, the chances of sister chromatid reunion are lessened by an increase in the distance between the two strands, and restitution is again the more common occurrence.

This point of maximum sensitivity has been suggested previously (SAX 1940; MULLER 1940), but chiefly on theoretical grounds and with little supporting evidence. Later, SAX and SWANSON (1941) showed that the peak of sensitivity of Tradescantia microspores was at a point at least eight hours after the chromosome split, and thus seemed to be due to some other factor.

Five-day Peak of Sensitivity.—The other peak of sensitivity was shown to be at about five days before anther dehiscence. This apparently represents some stage during the pollen grain mitosis. An examination of the types of aberrations produced at this stage (table 1) shows that almost all are either acentric fragments or dot deletions, the two types being present in about equal numbers.

Three slides prepared with pollen from flower No. 3 were consistent among themselves but were quite different from the other two flowers on that same day. The types of aberrations showed a low frequency of acentric fragments, and in general were typical of those found on the fourth day. Experience has

shown that there is some variation in the time required for buds to develop from the pollen grain mitosis to the dehiscence of the anthers. Flower No. 3, therefore, is considered to have developed more slowly than the two other flowers of the fifth day, and to represent a bud which was at a later stage of development when X-rayed. For that reason it is not classified with the other two flowers on the fifth day.

As shown by the sharp drop in the aberrations on the sixth and seventh days, abnormalities induced by X-rays at stages previous to the pollen grain division cannot be detected at the pollen tube division, because fragment loss prevents normal germination and growth of the pollen. As this fragment loss is only achieved when the chromosomes are X-rayed in a non-condensed state and immediate breakage is possible, the seventh and sixth days must represent stages from resting stage up to about mid-prophase respectively. The range of sensitivity during this period could not be determined by the technique used in this experiment. This, however, has been studied in detail (SAX and SWANSON 1941) and it has been found that the sensitivity, when based on the criterion of immediate chromosome breakage, steadily increases to a maximum about mid-prophase. The range in sensitivity was shown by them to be approximately 1 to 3.

At the five day stage a noticeable feature was the variability between slides and between the cells of a single slide. About 55 percent of the cells from flowers No. 1 and No. 2 showed no breaks at all or only one per cell, yet as many as 16 breaks were found in other cells. 13.3 percent of the cells showed more than eight breaks, with a mean of 11.5 ± 0.4 breaks per cell. The mean for the cells with less than eight breaks was 1.27 ± 0.1 breaks per cell, while the mean for all the cells was 2.58 ± 0.3 breaks per cell. On the basis of these facts, this range of sensitivity cannot be explained as random variation within the cells of a group due to chance factors alone (The Chi-square from a comparison of the high and low sensitivity groups is highly significant.) The most reasonable explanation for these differences is the variability in the stages of cells within a single anther at the time of irradiation.

Though the entire contents of an anther pass through the pollen grain division during one day, an examination of the cells at one time will show the nuclei to be in all stages of division. KOLLER (1943) has shown that there are seldom more than 50 percent of the cells in an anther undergoing the pollen grain mitosis (all stages) at any one time. About 60 percent to 75 percent of these are in prophase stages, and 12 percent to 18 percent at metaphase. Thus in the cells of an anther at this time one would expect to find about 50 percent resting stages, 35 percent prophase stages and 15 percent metaphase and anaphase stages. If there is a differential in sensitivity of the several stages, the frequency of the cells which show the greatest number of chromosome breaks will vary depending upon which stage is the most sensitive. This frequency of affected cells should roughly correspond to the percentages of the cells in that particular stage.

In the cells that were treated on the fifth day before anther dehiscence, analysis showed some 13 percent of the cells to have a significantly higher

sensitivity than the remainder. This would seem to indicate that some short period, possibly metaphase-anaphase, has an abnormally high sensitivity. That this stage is prophase is unlikely because (1) the percentage of very sensitive cells is too small, and (2) the degree of sensitivity is much greater than has been shown with techniques which accurately test the sensitivity of the prophase stage (SAX and SWANSON 1941).

Range of Sensitivity.—On the basis of the above argument it is considered that the maximum chromosome breakage shown on the fifth day is at the metaphase-anaphase stage of the pollen grain mitosis, and that its actual sensitivity can best be judged by the 13 percent of the cells which showed an abnormally high number of chromosome breaks. The break frequency in this group was 184 breaks in 96 chromosomes, or 192 percent. As a contrasting stage of low sensitivity, on the fourth day the cells were all in the post-mitotic resting stage, and showed a sensitivity of 18.5 percent chromosome breaks. This thus represents a range in sensitivity of about 1 to 10 between the least and most sensitive stages. Previous investigations with Tradescantia (SAX and SWANSON 1941) on the sensitivity of chromosomes to immediate breakage when X-rayed during the prophase of the pollen grain division, revealed a sensitivity range of 1 to 3. BOZEMAN and METZ (1949) working with aberrations detectable in the salivary glands of Sciara have shown a range of sensitivity of 1 to 10 between prophase and mid-anaphase. Because the resting stage is almost completely insensitive, the range through a complete cell division cycle would be considerably greater. Recent work with Allium (CONGER 1947 and unpublished), however, has shown a range of 1 to 37, and with Trillium (SPARROW 1948) a range of 1 to 50. The latter figure is based on meiotic divisions.

During the prophase peak of sensitivity on the third day, the cells are uniform in stage because of the long prophase development before the pollen tube division (about three days after doubling occurs compared with 24 to 36 hours for the prophase of the pollen grain division). For this reason, the whole group of cells from one flower should be considered in any measure of their sensitivity at this stage. This secondary maximum showed a break frequency of 45.6 percent (table 2 flower No. 1). Thus the range of sensitivity between the resting stage and prophase maximum at the time of chromosome doubling is 1 to 2.4.

It is probable that both of these ranges of sensitivity are actually greater than the figures above would indicate. Because the resting stage following the pollen grain division is of relatively short duration, its sensitivity is perhaps nearer that of an early prophase than that of a more prolonged resting stage. If instead, the sensitivity of the interphase between meiosis and the pollen grain division were used for comparison, it is probable that its aberration frequency would have been lower, and the sensitivity changes correspondingly greater.

Factors Affecting Aberration Frequency.—An examination of table 1 will show that the increase in sensitivity on the third day was produced chiefly by an increase in the number of acentric fragments, excluding dot deletions. On the fifth day (flowers No. 1, No. 2) it was due to an increase in both acentric

fragments and dot deletions, which occurred in about equal numbers. In each case some of the acentric fragments showed sister chromatid reunion at either one or both ends of the fragment. The degree of reunion on the two days is given in table 3.

From this it will be noted that on the third day practically all of the fragments showed union at least at one end. In each of the five cases that did not, the broken end of the centric fragment of the chromosome was found to have undergone sister chromatid reunion, thus preventing restitution. On the fifth day only about 50 percent of the fragments were found with sister chromatid reunion, and none was found with fusion at both ends.

TABLE 3

Degree of sister chromatid reunion during the prophase of the pollen tube division.

TYPE OF REUNION	3 DAYS			5 DAYS			
	NO. 1	NO. 2	TOTAL	NO. 1	NO. 2	NO. 3	TOTAL
0	6	16	22	0	0	0	0
U	18	16	34	9	2	0	11
‖	5	0	5	9	0	4	13
			61*				24*

* Based on relatively few fragments, as the types of many are difficult to determine. The types of reunion symbols indicate fusion at both, one, or neither end.

The above figures seem to indicate that the lateral fusion of sister chromatids at the three-day stage prevented the restitution of X-ray induced breaks, and was thus the primary cause of the high frequency of fragment formation on that day.

With chromosomes which were X-rayed on the fifth day, sister chromatid reunion cannot be an immediate cause of the failure of the broken ends to undergo restitution, because at that stage the chromosomes are still single stranded (at least to X-rays). Since prophase sensitivity is not detectable at the pollen tube metaphase, these cells of high sensitivity must represent a stage between late prophase and anaphase. During this period the chromosomes are in a condensed form. Within the matrix the chromonema is in a coiled condition, and likely under considerable tension due to the changes connected with the spiralization cycle. This force is probably longitudinal in the chromonema, and may be either one of contraction if the chromonema is becoming continually shorter as found in Trillium (SPARROW, HUSKINS, and WILSON 1941), or one of compression if caused by the shortening of the chromosome pellicle as postulated for Tradescantia (SAX and HUMPHREY 1934).

A break in a chromonema under stress would result in either the two ends separating from or slipping past one another in response to the coil tension. Either would cause the separation of the broken ends, thus preventing immediate restitution. The rigidity of the matrix which would prevent free move-

ment of the broken ends during the condensed state of the chromosomes, combined with the tension at anaphase due to the action of the spindle mechanism, may be able to maintain the breaks until spatial relationships decrease the chances of restitution or illegitimate fusion to a very low figure. Thus, at metaphase-anaphase, in direct contrast to the cause of the high sensitivity at the prophase maximum, the important factor is lack of fusion rather than illegitimate fusion (sister chromatid reunion).

DARLINGTON and LACOUR (1945) have attempted to explain the changes in sensitivity to X-ray breakage on the basis of chemical changes in the chromosomes, rather than any physical alterations as outlined above. Any stage during which the chromosomes are charged with nucleic acid, is considered by them to be strongly resistant to X-ray destruction, either because in that state the chromosomes cannot be broken, or the breaks when made immediately undergo restitution. On this basis, the late prophase, metaphase, and anaphase stages must be much less sensitive to X-ray breakage than the resting stage. This theory is not supported by the results of this research, the data suggesting just the reverse relationship of sensitivity to chromosome structure. BOZEMAN and METZ (1949) have found evidence in their work with Sciara that an increase in chromosome sensitivity is closely connected with the breakdown of the nuclear membrane. They postulate a cytoplasmic factor which conditions the sensitivity of the chromosomes, particularly during the condensed state, and suggest that the progressive increase in sensitivity after the nuclear membrane breakdown is due to the greater penetration of the substance through the nucleoplasm to the chromosomes, and the fact that it requires time to produce its maximum sensitizing effect. Because of the size of the chromonemata, they believe that any breakage is due to secondary effects such as stress from chromosome movement on weakened spots in the chromosomes caused by the X-ray ionizations.

The present research indicates that one point of high sensitivity is at the time of chromosome doubling during prophase, and in Tradescantia this occurs a considerable period of time before the breakdown of the nuclear membrane. The sharp increase later at metaphase-anaphase coincides with the disappearance of the nuclear membrane, but there is no evidence that it is a direct result of that change.

Refusion of Broken Ends.—That broken ends remain for a considerable length of time in a state capable of refusion with other broken ends, is shown by the fact that the acentric fragments induced at the metaphase-anaphase maximum showed sister chromatid reunion. Since these fragments were single stranded when the breaks were induced and remain so for about two days before chromosome doubling during the prophase of the generative nucleus division, the ends must remain in a fusable condition for at least that length of time.

Previous experiments with fractionated dosages (SAX 1939) indicated that in Tradescantia the broken ends of the chromosomes X-rayed at a stage five days before the pollen grain division were capable of refusion for a maximum period of about one hour. More recently SWANSON (1947) has been able to dem-

onstrate that recombination of broken ends in Tradescantia chromosomes may be affected for at least four hours after X-irradiation. McCLINTOCK (1939) found that a chromosome in maize broken at an anaphase bridge would undergo sister chromatid reunion when the chromosome doubled, and thus would form another bridge at the next anaphase. This condition persisted in the endosperm, but in the embryo the break healed and was no longer capable of sister reunion.

SAX (1941) concluded that sister chromatid reunion was caused by the fusion of two broken ends produced by the rupture of a double-stranded chromosome from the action of a single X-ray "hit," and found that it was only in very rare cases that the two broken ends of the chromatids failed to fuse. DARLINGTON and LACOUR (1945) consider that most sister chromatid reunions result from breaks in single-stranded chromosomes. In this case the union must be produced either by the fusion of broken ends after doubling occurs, or by the failure of terminal gene duplication. The theory has been criticized by CATCHESIDE (1948) on the grounds that the delay of chromatid union would cause the aberration frequency to be independent of the dosage intensity.

The present data suggest that sister chromatid reunion may result from breaks induced both before and after doubling. During a period lasting for at least two days before the chromosomes become effectively double to X-rays, any simple breaks induced in the single-stranded chromosome may result in isochromatid fragments. In about 50 percent of the fragments examined, sister chromatid reunion was found to have occurred after chromosome doubling had taken place.

On the other hand, simultaneous breakage of both chromatids at the time of chromosome doubling, was considered to be the cause of one of the peaks of X-ray sensitivity. This produced centric and acentric isochromatid fragments, in all cases of which at least one set of broken ends, and usually both, underwent sister chromatid reunion.

The fact that only about half of the isochromatid fragments, induced when the chromosomes were single stranded, showed sister reunion (table 3) would seem to indicate either that the union was not due to a failure of terminal gene duplication, or that in many cases the duplication process was normal. The alternative theory need only postulate that the broken ends remain in a state capable of fusion with another broken end. The present research cannot resolve this problem, but does suggest that this latter theory is perhaps the more reasonable of the two.

Though sister chromatid reunion is an important factor in the prophase sensitivity at the time of chromosome doubling in the generative nucleus of Tradescantia, it is quite possible that it is not of very widespread importance. Prophase stages of the pollen grain division in Tradescantia show no evidence of such a peak of sensitivity. This might be explained by a more rapid development and non-synchronization of stages, but in the pollen of Tulipa in which prophase splitting is nine days before dehiscence of the anthers, there is no sister chromatid reunion, but an increase in sensitivity is still found throughout

the prophase stage (NEWCOMBE 1942). Other factors, unidentified or unsuspected at present, *e.g.* differential primary breakage, may be of great importance in addition to the purely physical conditions considered above.

SUMMARY

A detailed analysis has been made of the changes in sensitivity of the chromosomes of Tradescantia to X-ray breakage during the mitotic cycle of the pollen grain division. The data obtained indicate:

1. There is a period of low sensitivity during the postmitotic resting stage, and two points of high sensitivity, one at the time of chromosome doubling during the prophase of the pollen tube division and the other at metaphase-anaphase of the pollen grain division, the latter being the greater of the two.

2. The range of sensitivity represented by the prophase maximum is 1 to 2.4, and the range at metaphase-anaphase 1 to 10, when the post-mitotic resting stage is considered as unity.

3. It is suggested that the prophase peak of sensitivity is at a point just before the chromosomes become "effectively double" to breakage by X-rays, but just after actual chromosome doubling has occurred. At that time the chances of sister chromatid reunion are almost, if not equal to the chances of restitution. At later stages the chances of restitution are much the greater.

4. It is suggested that the metaphase-anaphase peak of sensitivity is due to the prevention of restitution by (a) factors of longitudinal tension induced within the chromonema by changes during the spiralization cycle and later by the spindle mechanism, and (b) the chromosome matrix which reduces fragment movement after breakage and separation.

5. X-ray breaks induced during the stages before the prophase of the generative nucleus division are shown to remain open and to be capable of sister chromatid reunion when the chromosome becomes double, which may be as long as two days after the X-ray treatment.

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LITERATURE CITED

BISHOP, CHARLES J., 1949 Pollen tube culture on a lactose medium. Stain Techn. **24:** 9–12.

BOZEMAN, M. L., 1943 Effects of irradiation on oöcytes of Sciara. Genetics **28:** 71 (abstract).

BOZEMAN, M. L., and C. W. METZ, 1949 Further studies on sensitivity of chromosomes to irradiation at different meiotic stages in oöcytes of Sciara. Genetics **34:** 285–314.

CARLSON, J. GORDON, 1942 Immediate effects of 31r of X-rays on the different stages of mitosis in neuroblasts of Chortophaga. J. Morph. **71:** 449–462.

CATCHESIDE, D. G., 1948 Genetic effects of radiations. Advances in Genetics **2:** 271–358.

CONGER, ALLEN, 1947 X-ray sensitivity of the stages of mitosis. Amer. J. Bot. **34:** 582 (abstract).

CREIGHTON, MARGARET, 1941 The effects of X-rays on mitosis and meiotic chromosomes and cell division in *Chorthippus longicornis* (Orthoptera). J. exp. Zool. **87:** 347–369.

DARLINGTON, C. D., and L. F. LACOUR, 1945 Chromosome breakage and the nucleic acid cycle. J. Genet. **46:** 180–267.

HOLTHUSEN, HERMANN, 1921 Pflügers Archiv. **187:** 1–24.

KOLLER, P. C., 1943 The effects of radiation on pollen grain development, differentiation, and germination. Proc. roy. Soc. Edinb. **61:** 398–429.

KRAUS, P. and K. ZIEGLER, 1906 Experimentelle Untersuchungen über die Einwirkung der Röntgenstrahlen auf tierisches Gewebe. Fortschr. Röntgenstr. **10:** 126–182.

MCCLINTOCK, BARBARA, 1939 The behavior in successive nuclear divisions of a chromosome broken at meiosis. Proc. nat. Acad. Sci. **25:** 405–416.

MOTTRAM, J. C. 1913 On the action of beta and gamma rays of radium on the cell in different states of nuclear division. Arch. Middlesex Hosp. **30:** 98–119.

MULLER, H. J., 1940 An analysis of the process of structural change in the chromosomes of Drosophila. J. Genet. **40:** 1–66.

NEWCOMBE, H. B., 1942 The action of X-rays on the cell. I. The chromosome variable. J. Genet. **43:** 145–171.

REYNOLDS, J. PAUL, 1941 X-ray induced chromosome rearrangements in the females of Sciara. Proc nat. Acad. Sci. **27:** 204–208.

RICK, CHARLES M., 1940 On the nature of X-ray induced deletions in Tradescantia chromosomes. Genetics **25:** 467–482.

SAX, KARL, 1939 The time factor in X-ray production of chromosome aberrations. Proc. nat. Acad. Sci. **25:** 225–233.

1940 An analysis of X-ray induced chromosomal aberrations in Tradescantia. Genetics **25:** 41–68.

1941 Types and frequencies of chromosomal aberrations induced by X-rays. Cold Spr. Harb. Symp. Quant. Biol. **9:** 93–101.

SAX, KARL, and L. M. HUMPHREY, 1934 Structure of meiotic chromosomes in microsporogenesis of Tradescanta. Bot. Gaz. **96:** 353–361.

SAX, KARL, and C. P. SWANSON, 1941 Differential sensitivity of cells to X-rays. Amer. J. Bot. **28:** 52–59.

SPARROW, ARNOLD H., 1944 X-ray sensitivity changes in meiotic chromosomes and the nucleic acid cycle. Proc. nat. Acad. Sci. **30:** 147–155.

1948 Relative X-ray sensitivity of metaphase and interphase chromosomes. Nature **162:** 651.

SPARROW, ARNOLD H., HUSKINS, C. L., and G. B. WILSON, 1941 Studies on the chromosome spiralization cycle in Trillium. Canad. J. Res. **20:** 257–266.

SWANSON, C. P., 1940 A comparison of chromosomal aberrations induced by X-ray and ultraviolet radiation. Proc. nat. Acad. Sci. **26:** 366–373.

1947 The effect of infrared treatment on the production of X-ray induced changes in the chromosomes of Tradescantia. Amer. J. Bot. **34:** 590 (abstract.)

WHITING, ANNA R., 1940 Sensitivity to X-rays of different meiotic stages in unlaid eggs of Habrobracon. J. exp. Zool. **83:** 249–269.

1945a Effects of X-rays on hatchability and on chromosomes of Habrobracon eggs treated in first meiotic prophase and metaphase. Amer. Nat. **79:** 193–227.

1945b Dominant lethality and correlated chromosome effects in Habrobracon eggs X-rayed in diplotene and in late metaphase I. Biol. Bull. **89:** 61–71.

A CORRELATION OF PHENOTYPIC CHANGES AND CHROMOSOMAL REARRANGEMENTS AT THE TWO ENDS OF AN INVERSION[1]

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IF THE phenomenon of position effect in Drosophila is to be interpreted to mean a change in the activity of a gene due to a change of its position on the chromosome, there are several methods by which the validity of the hypothesis may be tested. One such method has been to start with a chromosomal rearrangement which is known to produce a position effect and experimentally alter the rearrangement so as to obtain reversions of the effect. If the activity of a gene is dependent upon its position in the sequence of genes, then it is logical, according to the hypothesis, that when the gene is moved to a new position, its activity may be altered, resulting in a changed phenotype. It then follows, that if the gene is moved back to its original position in the sequence, it should resume its original activity. This result was obtained in the case of a spontaneous reinversion (GRÜNEBERG 1937), and when the original order was restored by means of crossing over (ZELENY 1919; STURTEVANT 1925; DUBININ and SIDOROV 1935; PANSHIN 1935).

It was still not known whether it was necessary to move the gene back to its original position in order to restore its original activity, or if it could carry on its original activity in some other positions. That a gene would have its original activity in a number of positions on the chromosome was demonstrated by the cytological study of reversion of position effect phenotypes to the wild type (GRIFFEN and STONE 1940; KAUFMANN 1942; SUTTON 1943).

The evidence strongly indicated that many position effects were caused, not by removing a gene from its usual position in the sequence, but rather by bringing it into the proximity of heterochromatin (SCHULTZ 1936; SCHULTZ and CASPERSSON 1939; DEMEREC 1940, 1941; KAUFMANN 1942). Numerous cases were found in which one break was in heterochromatin (summarized by WHITE 1945, p. 67; listed by BRIDGES and BREHME 1944). GRIFFEN and STONE (1940), however, took exception to this view from their data on a white mottled eye Drosophila stock.

Data were also forthcoming to indicate that the quantity of heterochromatin as well as the proximity played a role in position effect (PANSHIN 1938, KAUFMANN 1942); and also that the quality of the heterochromatin was a variable (PANSHIN 1938; KHVOSTOVA 1941; DEMEREC 1941; KAUFMANN 1942).

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Probably the most thorough analysis of reversions from a position effect to wild type is that of KAUFMANN (1942) working with the roughest-3 inversion on the X chromosome of *Drosophila melanogaster*. However, there are certain disadvantages to the use of this inversion in a precise analysis of the role of heterochromatin, since the inversion places the roughest locus in a region of heterochromatin found by MULLER and GERSHENSON (1935) to consist of large blocks of heterochromatin, some of which do not appear on the salivary gland chromosome (HINTON 1942), and also in a region adjacent to the nucleolus organizing region and close to the kinetochore. The other end of the inversion is, unfortunately, located in a region suspected of containing some heterochromatin, the amount not having been determined. It is difficult to separate these various elements and their respective roles in the position effect.

Therefore, an attempt was made to circumvent the difficulty encountered by KAUFMANN in his analysis, by seeking for the present analysis an inversion in another chromosome with breaks in less complex regions.

As long as questions still remain concerning the relation of the gene to its position on the chromosome, the influence of heterochromatin on gene expression, the influence of the quantity and of the quality of the heterochromatin, and the possible roles of the nucleolus and of the kinetochore, further studies are needed. The present study was designed to test as many of these factors as possible.

Preliminary reports of this study have appeared (HINTON 1947, 1948a, 1948b, 1949a).

MATERIAL

An interbrachial inversion of the second chromosome of *Drosophila melanogaster* was selected for this study. The inversion was found from X-ray treated material by HINTON and ATWOOD (1941) and called by them *In(2LR)40d*.

The left break of the inversion is on the left arm of the chromosome in region 26D (BRIDGES' map) just before 26E. This is a region which behaves like and appears to be euchromatin. It is adjacent to prominent bands, as seen on the salivary gland chromosomes, which can easily be followed in subsequent rearrangements.

The right break is on the right arm at 41A/B. This is in a region of heterochromatin which takes a dark stain, is relatively compact, and lends itself to analysis better than most heterochromatic regions. This region of the salivary gland chromosome was located on the mitotic chromosomes by HINTON (1942) and was found to be distal to the heteropycnotic region around the kinetochore of the mitotic chromosome. The break is enough removed from the spindle attachment region so that heterochromatin can be observed on the salivaries both proximal and distal to the break.

When the section between the two breaks became inverted, it resulted in the euchromatic region 26D being adjacent to the heterochromatic 41A, and the euchromatic region 26E adjacent to the heterochromatic 41B. It was not known which genes were affected by the inversion. It could have been the genes

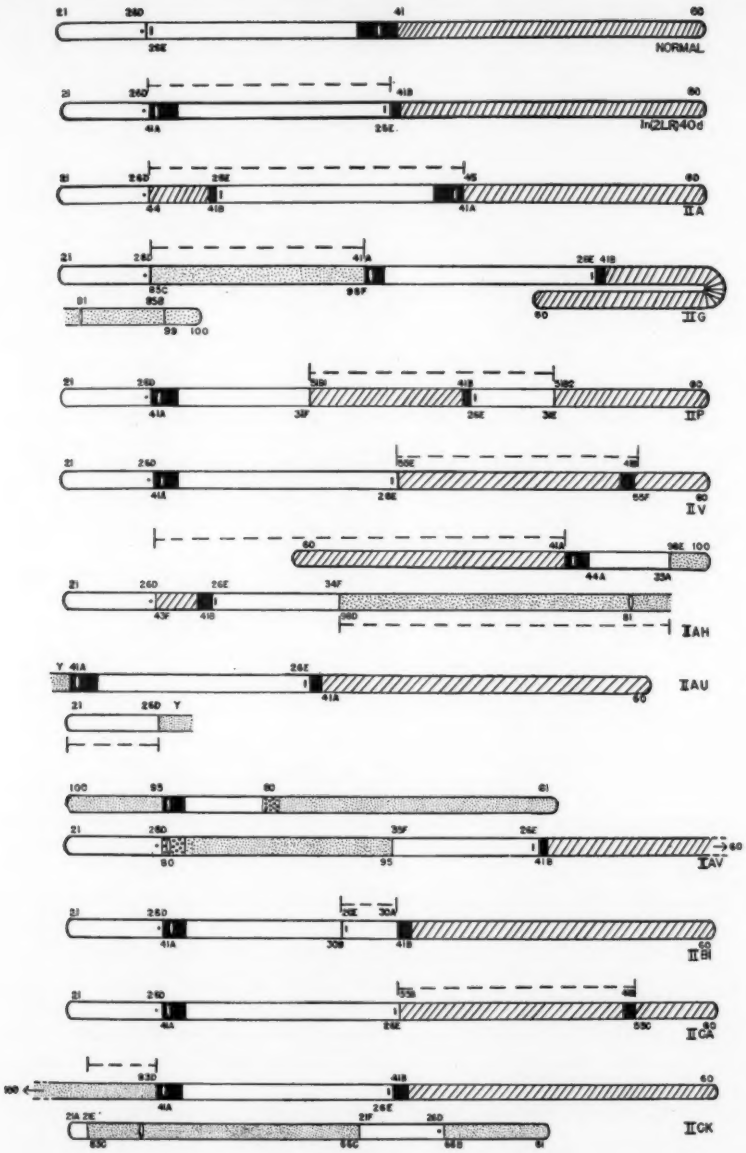

FIGURE 1

(*Continued on p. 192*)

in 26D or in 26E. For that reason, it was necessary to pay attention to both ends of the inversion in this study.

The phenotype caused by *In(2LR)40d* is an effect on the eye. It results in a roughening of the facets of the eye, a mottling of the pigment of the eye, and the occurrence of black spots of a tumor-like nature on the surface of the eye. The phenotype and the various environmental and genetic factors which influence its expression have been described in detail elsewhere (HINTON 1949b).

The inversion is lethal when homozygous.

PROCEDURE AND RESULTS

Males heterozygous for Inversion *(2LR)40d* were irradiated with X-rays at a dosage of 4000 r. The other second chromosome carried Bristle (*Bl*), a dominant gene which served as a marker. The treated males were mated to females heterozygous for *In(2LR)40d* and for Curly, brown, speck (*Cy bw sp*^{2}).

Since *In(2LR)40d* is homozygous lethal, three types of offspring were expected: *Bl/In(2LR)40d*, *Bl/Cy bw sp*^{2}, and *Cy bw sp*^{2}/the treated *In(2LR)40d*. As the first two type of offspring do not carry the treated inversion chromosome, they were discarded. The last type mentioned was examined in order to detect any changes in the characteristic appearance of the *In(2LR)40d* eye. Of this type, 3571 were examined and 105 individuals were selected as having a possible phenotypic change.

Each of the 105 possible cases of a phenotypic change was mated to flies from a balanced stock (*Cy/Pm;H/Sb C*) and, where possible, the treated second chromosome was subsequently carried in stock heterozygous with the Curly chromosome. In 46 cases the attempt to breed the fly showing the phenotypic change was unsuccessful. Thus 43.8 percent of the possible changes proved to be sterile or died without offspring.

The degree to which *In(2LR)40d* affects the appearance of the eye is variable and in some cases the eye is indistinguishable from a normal wild type eye. It was, therefore, to be expected that some of the 105 changes detected would

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Diagrams of the chromosomes from the various reversion stocks.

The regions originally to the the left and the right of the distal-most break of *In(2LR)40d* are represented respectively by a dot and a bar to facilitate locating them on the various diagrams.

The different chromosome arms are demarked as follows:

2L is drawn in outline only.
2R is crosshatched.
3L, *3R*, and *Y* are stippled.

The solid black represents the heterochromatic regions of the second chromosome; the large stipples, the heterochromatin of the third.

The numbers above and below the chromosomes correspond to the regions on BRIDGES' maps of the salivary gland chromosomes.

The Roman numerals and letters at the right of each chromosome indicate the experiment number of the reversion.

The broken line above, or below, the various chromosomes indicates the extent of the rearrangement. They are omitted in some of the more complex rearrangements.

The broken line at the end of some diagrams indicates that the chromosome extends beyond that point to the region denoted by the arrow.

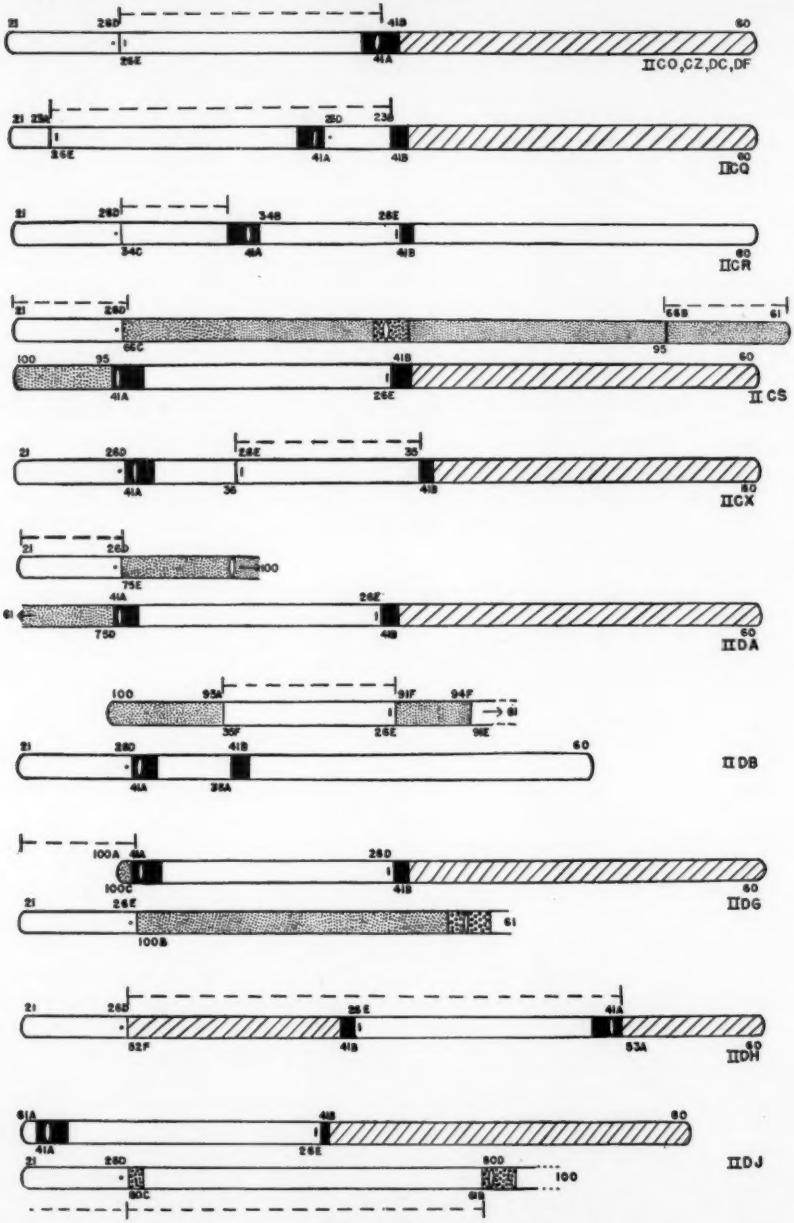

FIGURE 1 (*continued*)

be, in actuality, not changes but the unaltered inversion simulating wild type in its expression. Upon further breeding, 26 of the possible changes immediately gave offspring so similar to the usual *In(2LR)40d* type that they were discarded without further analysis.

The remaining 33 stocks were each analyzed cytologically by an examination of aceto-orcein smear preparations of the salivary gland chromosomes. In nine cases, it was found that no change in the chromosomes had occurred and since the phenotype of the flies had through the intervening time gradually changed to that shown by the untreated *In(2LR)40d*, it was concluded that these also had been merely a somewhat prolonged overlapping of the wild type and that they were unaltered *In(2LR)40d*. These nine stocks were, therefore, discarded.

The results of the cytological analysis of the remaining 24 stocks are presented below along with a description of the phenotype in each case, and are diagrammed in figure 1. The stocks are grouped according to the location of the new breaks on the chromosome. The number and letter preceding each description is the experiment number. The symbols following the experiment number summarize the description of the chromosomes according to the procedure of nomenclature as suggested by BRIDGES and BREHME (1944). The location of breaks is presented as numbers referring to the region on BRIDGES' map.

Cases Where One New Break Coincides with the Left Break of In(2LR)40d

II A *In(2LR)IIA*. An inversion in the second chromosome. One break separates region 26D from 41A. The other break occurs between region 44 and 45. Thus the new order on the chromosome is 21–26D, 44-41B, 26E-41A, 45-60.

The eye is very rough with some mottling of the pigment. However, no black spots occur, but some eyes show dark areas.

II G *T(2;3)IIG*. A section of the third chromosome inserted in the second. Breaks in 3R occur at 98F and at 85C. The piece of 3R between these breaks is inserted into 2 between 26D and 41A. Thus the new order on the chromosomes is 21-26D, 85C-98F, 41A-26E, 41B-60; 81-85B, 99-100.

The eye is completely wild type in appearance.

II AH *T(2;3)IIAH*. A translocation between the second and third chromosomes plus an inversion in the second. Breaks in 2 occur between 26D and 41A, between 43F and 44A, and between 34F and 35A. 3R is broken between 98D and E. The order of parts in the new arrangement is 60-44A, 41A-35A, 98E-100; 21-26D, 43F-41B, 26E-34F, 98D-81.

The eye is slightly rough; otherwise, it appears wild type.

II AV *T(2;3)IIAV*. A translocation between 2L and 3L and between 2R and 3R. Breaks in 2 occur between 26D and 41A and between 35E and F. 3L is broken at the chromocenter in region 80 and 3R is broken in region 95. The order of parts in the new arrangement is 21-26D, 80-95, 35F-26E, 41B-60; 61-80, 35E-41A, 95-100.

The eye is slightly to moderately rough with a few black areas but no true black spots.

II CK *T(2;3)IICK*. A reciprocal translocation between the tip of 2L and most of 3R plus a piece of 2L inserted in 3L. The breaks in 2 occur between 21E and F and between 26D and 41A. The break in 3R is between 83C and D. The break in 3L is between 66B and C. The order of parts in the new arrangement is 21A-21E, 83C-66C, 21F-26D, 66B-61; 100-83D, 41A-26E, 41B-60.

The eye is slightly to moderately rough with a few black spots occurring.

II CR *In(2LR)IICR*. An inversion in the second chromosome. One break separates region 26D from 41A. The other break occurs at 34C. The new order of parts of the chromosome is 21-26D, 34C-41A, 34B-26E, 41B-60.

The eye is completely wild type in appearance.

II CS *T(2;3)IICS*. A reciprocal translocation between the tip of 2L and the third chromosome, with a transposition in the third. The break in 2 occurs between 26D and 41A; the break in 3L at 66C; and the break in 3R at 95. The piece of 3L from the break to the tip is transposed to the base of 3R. The part of 3R from the break to the tip is translocated to the base of 2R, while the tip of 2L is translocated to the base of 3L. The order of parts on the two chromosomes is therefore 100-95, 41A-26E, 41B-60; 21-26D, 66C-95, 66B-61.

The eye is slightly to moderately rough with an occasional small black spot.

II DA *T(2;3)IIDA*. A reciprocal translocation between the tip of 2L and 3L. The break in 2 occurs between 26D and 41A; the break in 3L between 75D and E. The new order of parts is 61-75D, 41A-26E, 41B-60; 21-26D, 75E-100.

The eye is slightly rough, but there are no black spots.

II DG *T(2;3)IIDG*. A reciprocal translocation between the tips of 2L and 3R. The break in 2 is between 26D and 41A. The break in 3R is between 100B and C. Thus the new order of parts of the chromosomes is 100A-100C, 41A-26D, 41B-60; 21-26E, 100B-61.

The eye is slightly to moderately rough with occasional traces of dark areas but no black spots.

II DH *In(2LR)IIDH*. An inversion in the second chromosome. One break separates region 26D from 41A. The other break occurs between 52F and 53A. The order of parts on the rearranged chromosome is 21-26D, 52F-41B, 26E-41A, 53A-60.

The eye is very slightly rough but otherwise appears wild type.

II DJ *T(2;3)IIDJ*. A reciprocal translocation between the tips of 2L and 3L, and an inversion in 3L. The break in 2 separates regions 26D and 41A. The breaks in 3L are at 61A6 and between 80C and D. The new order of parts of the chromosome is 61A1-61A6, 41A-26E, 41B-60; 21-26D, 80C-61B, 80D-100.

The eye is completely wild type in appearance.

Cases Where One New Break Coincides with the Right Break of In(2LR)40d

II V *In(2LR)IIV*. An inversion in the second chromosome with one break between 26E and 41B and the other break between 55E and F. The order of parts in the rearranged chromosome is 21-26D, 41A-26E, 55E-41B, 55F-60.

The eye is almost wild type in appearance. There is a very slight rearrangement of the facets.

II BI *In(2LR)IIBI*. A small inversion in the second chromosome with one break between 26E and 41B and the other between 30A and B. The resulting order of parts of the chromosome is 21-26D, 41A-30B, 26E-30A, 41B-60.

The eye is entirely wild type in appearance.

II CA *In(2LR)IICA*. An inversion in the second chromosome with one break between 26E and 41B and the other between 55B and C. The new order of parts of the chromosome is 21-26D, 41A-26E, 55B-41B, 55C-60.

The eye is slightly to moderately rough. No black spots appear.

II CQ *In(2LR)CQ*. An inversion with one break between 26E and 41B and the other break between 23A and B. The resulting order of parts of the chromosome is 21-23A, 26E-41A, 26D-23B, 41B-60.

The eye is very rough with a few black spots and dark areas occurring.

II CX *In(2LR)CX*. An inversion with one break between 26E and 41B and the other break between regions 35 and 36. The order of parts on the rearranged chromosome is 21-26D, 41A-36, 26E-35, 41B-60.

The eye is completely wild type in appearance.

II DB *T(2;3)DB*. A section of 2L is inserted into 3R at one end of a short inversion in 3R. The breaks in 2 are between 26E and 41B and between 35F and 36A. 3R is broken between 91E and F and between 94F and 95A. The order of the rearranged chromosome parts is 21-26D, 41A-36A, 41B-60; 61-91E, 94F-91F, 26E-35F, 95A-100.

The eye is completely wild type in appearance.

Miscellaneous Cases

II AU *T(Y;2)IIAU*. A translocation between the Y chromosome and the second, determined from genetic results. The breaks have not been determined cytologically, but one is probably between 26D and 41A on the second chromosome.

The eye of the male is wild type in appearance.

II DD. No new rearrangement can be detected. Cytologically, it is indistinguishable from *In(2LR)40d*.

The eye is completely wild type in appearance.

II P. *In(2LR)IIP*. An inversion in the second chromosome but neither break coincides with the breaks of *In(2LR)40d* which it overlaps. The new breaks are between 31E and F and between 51B1 and B2. The order of parts of the chromosome is 21-26D, 41A-31F, 51B1-41B, 26E-31E, 51B2-60.

The eye was indistinguishable from wild type for one generation. It subsequently became indistinguishable from the rough eye with black spots of *In(2LR)40d*.

II CO, II CZ, II DC, II DF. Four cases in which the cytological picture is indistinguishable from the wild type.

The eye is indistinguishable from wild type except in a few flies of *IICZ* where the eye is slightly rough.

CONCLUSIONS AND DISCUSSION

The Two Ends of the Inversion

The 24 cases analyzed in the present study which show either a phenotypic or a chromosomal change are of a variety of types, and generalizations are difficult to make. Probably the most striking feature of the results is that precisely the same phenotypic changes occur regardless of which end of the original inversion is altered by chromosomal rearrangements.

Seven cases were analyzed which showed complete reversion to the wild type phenotype. Three of these were due to the removal of region 26D from region 41A. However, in the other four cases, that section of the chromosome was intact with no evidence of any alteration in that vicinity, but rather region 41B (15 salivary regions beyond) was removed from region 26E.

Since the two ends of the inversion can produce the same effect, it is natural to search for differences in the effects of the ends. Table 1 attempts to summarize the comparison of the two ends.

TABLE 1

A comparison of the rearrangements at the left end of Inversion (2LR)40d with those at the right end.

	LEFT (26D–/41A)		RIGHT (26E–/41B)		TOTAL
	NUMBER	% OF TOTAL	NUMBER	% OF TOTAL	
Number of rearrangements	11	(64.7)	6	(35.3)	17
complete reversion of phenotype	3	(27.3)	4	(66.7)	7
partial reversion of phenotype	8	(72.7)	2	(33.3)	10
rearrangement involving a translocation to chromosome 3	8	(72.7)	1	(16.6)	9
with complete reversion of phenotype	2		1		3
with partial reversion of phenotype	6		0		6
rearrangement involving an inversion in chromosome 2	3	(27.3)	5	(83.4)	8
with complete reversion of phenotype	1		3		4
with partial reversion of phenotype	2		2		4

Frequency of Alterations at each End of the Inversion

As seen in the preceding table, one possible difference between the rearrangements at the two ends of the inversion is that there are nearly twice as many cases involving the left end. However, a chi-square test gives a probability of 0.23 that one would obtain a fit to the expected 1:1 as bad or worse than this. Therefore, this difference is probably not significant. Likewise, there is probably not a significant difference in comparative proportions of complete versus partial reversions at the left and right ends ($P=0.12$). The difference between a complete and a partial reversion may be correlated with a difference in location of the breaks. Perhaps, in the case of a complete reversion the break occurs exactly between region 26D, or region 26E, and its adjacent hetero-

chromatin so as to completely separate them from each other. The effect of the heterochromatin is then entirely overcome and the phenotype becomes wild type. There would be an equal chance of a break occurring at either of these two spots. However, if the break occurred within the adjacent heterochromatin so that region 26D, or region 26E, was not entirely separated from all of the heterochromatin, the amount removed with 26D, or 26E, might determine the extent of the roughness of the eye. This would account for the category of cases in which the phenotype is partially reverted to a greater or lesser degree.

At the left end, the only visible heterochromatin is region 41A. However, between 41A and the kinetochore is a piece of heterochromatin, invisible on the salivary gland chromosome but comprising about one-fifth of the right arm of the mitotic chromosomes (HINTON 1942). This means that there is a large area adjacent to region 26D which could be broken in any of a number of places and make no perceptible difference on the salivary chromosome. Adjacent to region 26E (the right break) there is a piece of heterochromatin comprising about one-twentieth of the arm of the salivary gland chromosome but probably comprising as much as a fifth of the mitotic chromosome arm (assuming 2R is comparable to 2L) (HINTON 1942). Therefore, there is an approximately equal amount of heterochromatin adjacent to each of the two sections (26D and 26E), and it would be expected that breaks would occur with an equal frequency. If the difference in the frequency of partial reversions at the two ends of the inversion were to be considered significant, the interpretation would necessarily include the assumption of qualitative differences in the two regions of heterochromatin.

Frequency of Translocations Involving Each End of the Inversion

Reversions associated with rearrangements between the third chromosome and the left end of the inversion occur more frequently than do reversions associated with rearrangements between the third chromosome and the right end of the inversion. In eight of the 11 rearrangements involving the left end of the inversion, the third chromosome is also involved while the right end is involved with the third chromosome in only one case. A chi-square test comparing the results to a random distribution gives a probability of 0.025. This strongly suggests that only certain regions of the chromosomes will react with 26D to restore or partially restore it to normal activity, and that a different set of regions is capable of restoring 26E to normal activity. The two ends are not equally affected by other chromosome regions and hence are not exactly the same, despite similar phenotypic effects.

Regions of the Chromosomes Associated with the Breaks

If we examine the various regions adjacent to 26D and 26E in the different reversion and partial reversion stocks (fig. 2), the nonrandom occurrence of regions associated with each becomes still more striking. A comparison of these regions to a Poisson distribution of breaks, gives a probability of 0.00055. It may be noted that neither region 26D nor 26E is ever found in the X chromosome. Region 26E is associated with regions of 2L in three cases, while 26D is

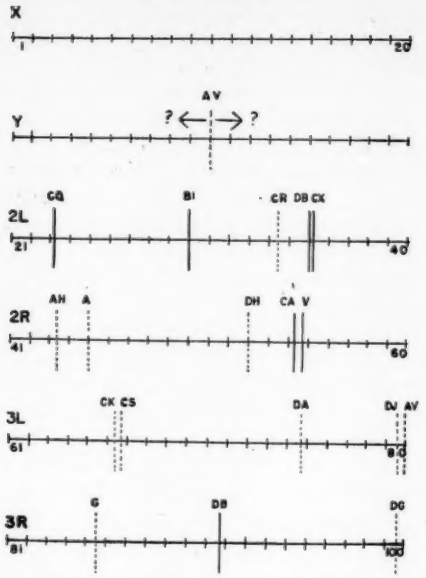

FIGURE 2. Diagrams of the salivary gland chromosomes to show the regions involved in the various reversions.

Each horizontal line represents a chromosome arm. Each is divided into 20 regions following the scheme of BRIDGES' maps.

The perpendicular lines denote the region adjacent to 26D or E in the particular rearrangement indicated by the letters. The solid lines indicate that the right end of Inversion (*2LR*)*40d* is involved in the rearrangement; the broken line indicates that the left end is involved.

found there only once. The reverse is true in 2R:26D, three times; 26E twice, but both times in the same section. In 3L, 26E does not appear; 26D appears five times, but in only three different sections. In no case are 26D and 26E associated with regions that are closer to each other than two map regions. Yet in region 55, 26E is associated with it in two cases; and regions 66 and 80 each have 26D associated in two cases. It can be concluded that not all regions of the chromosomes are equally effective in producing phenotypic changes of the *In*(*2LR*)*40d* eye, and different regions have an effect on each end of the inversion.

Reinversions

Four cases were found which appear to be complete reversions, cytologically and phenotypically. This unexpected result requires careful consideration due to its unprecedented nature. GRÜNEBERG (1936, 1937) reported a reversion to wild type of the *rst*^{3} inversion, but KAUFMANN (1942) was unable to obtain a repeat of the case even though he made a most thorough study of the inversion.

If reinversion of a chromosome can occur, it would indicate that the points of breakage of the original inversion were more sensitive to X-rays than was the rest of the chromosome. KAUFMANN (1939) was able to show that this was not true in the case of the delta-49 inversion, nor the *rst*^{3} inversion (1942). However, there are reports to the contrary (SITKO 1938a, 1938b; TSUBINA 1939, GRIFFEN and STONE 1940).

Unfortunately, the Inversion *(2LR)40d* chromosome carried no genetic markers or had any phenotypic effect other than the eye effect and a lethal effect. The most plausible explanation for these apparent reinversions would be contamination. Even though the occurrence of contamination cannot be disproved, it does appear unlikely for several reasons. In the first place, the four cases under consideration occurred in three separate experiments which were completed independently of each other. The fathers in each case were selected for rough eyes and were irradiated independently of the other groups. The mothers were selected for the marker traits on different occasions and came from different sets of bottles. Therefore, for contamination to be the explanation, it would have had to occur on three separate occasions by flies that were wild type in each case. No other contamination of any sort was found in any of the cultures. Of the four cases found, *II CO* was the only fly of its type in an experimental batch of 470 flies; *II CZ*, the only one of 674 flies; and *II DC* and *DF* the only ones of 240 flies. Ten males were used as fathers in each of these experiments.

Furthermore, since *II CZ* has retained some of the rough eye effect of *In(2LR)40d*, it makes contamination seem even less likely.

Lethals in the Reinversions

Probably the most significant evidence against contamination results from an analysis of the lethals carried by these flies. All except *II CZ* are homozygous lethal. The lethal carried by *II DF* is the same as a lethal carried by *In(2LR)40d*, because the combination *II DF/In(2LR)40d* is not viable. Thus, both *II CZ* and *II DF* have something in common with the *In(2LR)40d* chromosome as it existed before X-raying. *II DC*, which appeared in the same experiment with *II DF*, also carried a lethal, but not the same one as *II DF*. It is the same lethal, however, as is carried by *II CO* which was discovered independently. Curiously, *II CO* is lethal with Plum but *II DC* is not.

A search was made in the parental stock in this laboratory for the lethal carried by *II DC* and *II CO*, but without success. One hundred test matings were made of *II CO* to the *In(2LR)40d/Cy* stock, and no lethal combinations were found. If the lethal exists at all in the laboratory stock, it is very uncommon.

Taking into account all of the evidence presented, contamination as an explanation for the apparent reinversions becomes most unlikely. It would have required a coincidence of a number of very unlikely events.

If these four stocks are truly derived from the Inversion *(2LR)40d* stock, they raise the question of what is the nature of the lethal effect of an inversion.

For here it appears that two lethals remain after the disappearance of the inversion. One is the same as that of the inversion; the other appears to be a new one. Other evidence on this question has been obtained, such as SUCHE, PARKER, BISHOP, and GRIFFEN (1939), but a number of aspects of the problem remain unanswered.

Crossing Over as an Explanation of Reinversion

Another possible explanation for the occurrence of the four apparent reinversions might be found in the phenomenon of crossing over. A similar type of explanation for GRÜNEBERG's reinversion was offered by KAUFMANN (1942).

Theoretically, it is possible to obtain a triple crossover between Inversion *(2LR)40d* and the Curly inversions which would result in a chromosome with the wild type sequence of parts. However, only the females which were mated to the irradiated males came from a stock of that type, and the only offspring selected from them were ones having the Curly phenotype. The cytological examination shows that the Curly inversions are still present. As these must have been contributed by the original females, the possibility of their having contributed any other second chromosome is excluded.

The males to be irradiated were taken from a stock of *Bl/In(2LR)40d*. It would be possible, by a double crossover, to obtain from such a stock a chromosome lacking *Bl* and with the wild type sequence of parts. Occasional crossovers of this type have been observed in this laboratory in other experiments. Were a male resulting from such a crossover, and heterozygous for *Bl*, by chance selected for irradiation, his offspring would be of the type recovered in the four cases under consideration. However, all Curly, non-*Bl* offspring would be of this type. It would, therefore, be expected that a number of such offspring would be found together. As was previously stated, these offspring were the only example of that type in the entire culture in which they appeared. From 12 to 30 of this type would be expected if a crossover were the explanation.

None of the foregoing explanations seems very plausible. However, as long as these alternative explanations exist, it cannot be claimed with certainty that these are cases of reinversion of the chromosomes.

Phenotypic Reversion without Chromosomal Rearrangement

One case (*II DD*) was obtained in which the phenotype has reverted entirely to the wild type expression, yet there is no observable chromosome change. The original *In(2LR)40d* remains intact. If it is the heterochromatin adjacent to regions 26D and 26E that is responsible for the initial phenotype, and since in this case these regions are still adjacent to the heterochromatin, then some change in that heterochromatin supposedly has occurred. Since this change is not visible upon analysis of the salivary gland chromosome it may mean that, in some way, the heterochromatin has been inactivated or mutated. A case, somewhat comparable, was found for the Bar locus by SUTTON (1943), and at the brown locus (bw^D) by HINTON and GOODSMITH (in press). The possibility also exists that there has been an alteration within the heterochromatic regions which does not lend itself to study on the salivary chromosomes.

Removal to Other Regions of Heterochromatin

Two cases (*II AV* and *II DJ*) were found in which region 26D had been separated from the heterochromatin of 41A but had been moved adjacent to heterochromatin of the third chromosome. In one case the phenotype completely reverted to the wild type; in the other, only partial reversion had occurred. In both cases it was the same heterochromatin (region 80 of 3R). In all other cases studied region 26D or 26E was removed from the heterochromatin of region 41 by a rearrangement, which placed it next to supposedly euchromatic regions. In these two exceptional cases the indications are that not all heterochromatin behaves as does that in region 41. At least region 80 in these cases acts as a euchromatic region. Earlier in the discussion it was suggested that there was evidence for two types of heterochromatin—one, visible on the salivary chromosome and capable of exerting an inhibiting effect on regions 26D and E; the other, not visible on the salivary chromosomes but having an inhibiting effect. Region 80 appears to be a third type—visible on the salivary chromosome but exerting no inhibiting effect. Still a fourth type seems to comprise the Y-chromosome.

The Heterochromatin of the Y-chromosome

On genetic analysis, *II AU* is a translocation between the second and Y-chromosome. The eye is wild type in appearance even though the Y-chromosome is composed almost entirely of heterochromatin. From the cytological examination, it appears that region 26D is brought by the translocation to lie adjacent to part of the Y-chromosome. Such an analysis is difficult, however, since the Y-chromosome is practically invisible in salivary gland preparations. Nevertheless, a fourth type of heterochromatin is suggested—invisible on salivary chromosomes and having no inhibiting effect on region 26D. It was suggested from work with extra Y-chromosomes (HINTON 1949b) that they could suppress the inhibiting effect of the heterochromatin of region 41A.

A Rearrangement Overlapping In(2LR)40d

A case was analyzed (*II P*) in which an inversion on the second chromosome was present, overlapping the original inversion (*In(2LR)40d*), but not coinciding with either of the original breaks. The position of region 26D remains unaltered; the position of 26E is changed in relation to the end of the chromosome but not in relation to the adjacent heterochromatin (41B). As would be expected, if the proximity of heterochromatin is the causal agent in the production of the *In(2LR)40d* phenotype, this new arrangement should not change the phenotype. In the stock at present, the phenotype of the flies is indistinguishable from that of *In(2LR)40d* flies. However, in the first generation from the original female, the offspring had wild type eyes, with one exception. In the second generation only about half of the offspring were wild type in appearance. It is conceivable that some original rearrangement became altered due to crossing over, or that some temporary inhibition of the effect of the heterochromatin occurred when the chromosome was hit with X-rays. In

either case, as the stock exists now, it offers further evidence that this position effect is due to the proximity of certain regions to heterochromatin and not to the relation of those regions to the end of the chromosome or to the kinetochore.

Frequency of Reversion

The frequency of reversion occurring in all gametes tested is calculated to be approximately 1.8 percent, using the method of calculation described by KAUFMANN (1942). By this method, KAUFMANN calculated that reversion of roughest-3 was occurring in about 0.4 percent of the gametes. Since the percentage for *In(2LR)40d* is over four times as high as that for *rst*^{3}, a more exact comparison of the two sets of data is warranted. A chi-square test comparing the two sets of data gives a probability of less than 0.0001. Taking the figures given by KAUFMANN (1942) for *rst*^{3} reversions and converting them into percent of flies analyzed and comparing them to the comparable figures for *In(2LR)40d* reversions, a number of similarities are obtained. KAUFMANN analyzed 72 cases; the present study, 105. Of those KAUFMANN found 44.4 percent were sterile or inviable; the present study, 43.8 percent. KAUFMANN found that 32.0 percent simulated wild type owing to variability of expression; the present study, 33.3 percent. KAUFMANN found 23.6 percent of them were true reversions; 22.8 percent in the present study. These figures are so similar as to give a false picture. Actually, KAUFMANN's figures represent a much smaller percent of the total flies examined (21,104) and the present figures a much higher percent (3,571 flies examined). It means that a higher percentage of the flies examined in the present study were selected as being possible reversions, but that of those that were selected, the percentages of sterility, "false alarms," and actual reversions were the same as among those selected by KAUFMANN.

The high frequency of reversions found in the present study is probably due, at least in part, to the fact that breaks were recovered at both ends of the inversion.

In(2LR)40d as a Position Effect

In light of previous analyses of position effects, it was reasonable to assume *a priori* that some gene or genes affecting the development of the eye were inhibited in their action by being placed adjacent to heterochromatin as a result of the inversion. If it were a single gene, then only the heterochromatin at one end of the inversion should exert this inhibition. Since the phenotype, in this case, is at least twofold (the roughening of the facets, and the black tumorlike protuberances or spots) it was thought that two genes were possibly involved—one for each of the effects. Two such recessive genes are known in this general area of the chromosome (clot and pied) (HINTON and ATWOOD 1941). Therefore, it was assumed that either both of these genes were affected by the heterochromatin at one end of the inversion, or that one of the chromosome breaks had occurred between the two genes and each was affected by a different end of the inversion. If the first of these possibilities were true, the entire phenotypic effect would be expected to disappear upon altering one end, and only one end, of the inversion. If the alternative possibility were true, the roughen-

ing effect should disappear when one end of the inversion was altered; the black spots, when the other end of the inversion was altered.

The results of the cytological study show that none of the foregoing assumptions are valid and that this classical interpretation of position effect is not sufficient to explain the present data. The entire phenotypic effect may be caused to disappear upon alteration of either end of the inversion. The black spots may be caused to disappear upon alteration of either end of the inversion without the roughening effect being affected; but, the roughening effect cannot be caused to disappear without also having the black spots disappear. This is true regardless of which end of the inversion is altered. Presumably, then, the black spots are simply an extreme developmental effect of the rough phenotype and are not at all independent of it.

The present situation, therefore, cannot be interpreted as a simple effect of heterochromatin upon individual genes. It may mean that we are dealing here with a region of the chromosome which is a duplication, the two parts of which are normally concerned with the production of a normal eye, either of which alone can accomplish the same end. In this case it would have to be assumed that the two parts of the duplication were separated by one break of the inversion and each part was inhibited by being placed adjacent to heterochromatin. It would further have to be assumed that the phenotype may be restored or partially restored to the normal if either of these two parts is subsequently removed from its position adjacent to heterochromatin, since each part can function independently of the other.

It has been called to the author's attention that DUBININ (1936) working with the Plum inversion, likewise found that phenotypic changes could be produced by altering either end of that inversion.

If the hypothesis of a duplication can be shown to be untenable, then one must conclude that position effect is more complicated than it is supposed to be at present. The phenomenon would have to be interpreted as some overall effect upon the metabolism of the entire chromosome correlated with the position of the heterochromatin upon the chromosome.

SUMMARY

Males of *Drosophila melanogaster*, carrying an inversion in the second chromosome which causes an effect on the eye, were treated with X-rays. Among the offspring, individuals were selected which showed changes in the phenotype of the eye. Twenty-four such changes were successfully acquired and each was analyzed cytologically from salivary gland chromosome preparations.

Some phenotypic changes were complete reversions to the wild type phenotype, while others were only partial. Most of them proved upon analysis to be associated with chromosomal rearrangements.

Some of the chromosomal rearrangements involved the left end of the original inversion, while others involved only the right end. The same types of phenotypic changes occurred in both cases. The degree of reversion is correlated with a quantitative effect of heterochromatin.

The regions of the chromosomes capable of overcoming the position effect are not the same ones for the left end of the inversion as for the right. This is shown by a much greater frequency of translocations with the third chromosome in reversions associated with breaks at the left end of the inversion.

The distribution of reversion-effecting breaks along the several chromosomes is shown not to fit a Poisson series. This indicates that not all regions of the several chromosomes are equally effective in changing the *In(2LR)40d* phenotype when placed adjacent to it by rearrangements following irradiation.

Four cases of complete reinversion were obtained. Contamination as an explanation was ruled out. Crossing over, though not plausible, remains as a possible explanation. The lethal nature of the reinversions is discussed.

The data show that phenotypic change also may occur without altering the preexisting inversion; and that the preexisting inversion may be altered without a phenotypic change.

The total frequency of reversions was found to be significantly higher than in previous work by other authors.

Four types of heterochromatin are suggested by the differences in effect manifested on this position effect.

The significance to the understanding of position effect of altering both ends of the aberration is considered.

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LITERATURE CITED

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552:** 1–257.

DEMEREC, M., 1940 Genetic behavior of euchromatic segments inserted into heterochromatin. Genetics **25:** 618–627.

1941 The nature of changes in the white-Notch region of the X-chromosome of *Drosophila melanogaster*. Proc. 7th Int. Cong. Genetics 99–103.

DUBININ, N. P., 1936 A new type of position effect. Biol. Zh. **5:** 851–874.

DUBININ, N. P., and B. N. SIDOROV, 1935 The position-effect of the hairy gene. Biol. Zh., **4:** 555–568.

GRIFFEN, A. B., and W. S. STONE, 1940 The w^{m5} and its derivatives. Studies in the genetics of Drosophila. Univ. Texas Publ. **4032:** 190–200.

GRÜNEBERG, H., 1936 A case of complete reversion of a chromosomal rearrangement in *Drosophila melanogaster*. Nature **138:** 508.

1937 The position effect proved by a spontaneous reinversion of the X-chromosome in *Drosophila melanogaster*. J. Genet. **34:** 169–189.

HINTON, TAYLOR, 1942 A comparative study of certain heterochromatic regions in the mitotic and salivary gland chromosomes of *Drosophila melanogaster*. Genetics **27:** 119–127.

1947 An analysis of rearrangements of chromosomal aberrations producing position effects. Rec. Genetics Soc. Am. **16:** 35. (Also Genetics **33:** 108).

1948a The influence of various factors on the expression of a position-effect. Anat. Rec. **101:** 21.

1948b Analysis of chromosomal rearrangements affecting the expression of a position-effect. Rec. Genetics Soc. Am. **17:** 41. (Also Genetics **33:** 612).

1949a The role of heterochromatin in position effect. Proc. 8th Int. Cong. Genetics 595–596.

1949b The modification of the expression of a position effect. Amer. Nat. **83**: 69–94.

HINTON, TAYLOR, and K. C. ATWOOD, 1941 Position effect; terminal adhesions. Carnegie Inst. Wash. Yrbk. **40**: 231–232.

HINTON, TAYLOR, and W. GOODSMITH, 1950 An analysis of phenotypic reversion at the brown locus in Drosophila. J. exp. Zool. (in press).

KAUFMANN, B. P., 1939 Distribution of induced breaks along the X-chromosome of *Drosophila melanogaster*. Proc. nat. Acad. Sci. **25**: 571–577.

1942 Reversion from roughest to wild type in *Drosophila melanogaster*. Genetics **27**: 537–549.

KHVOSTOVA, V. V., 1941 The influence of the proximal chromosome regions on the position effect of the cubitus interruptus gene in *D. melanogaster*. D. I. S. **15**: 25–26.

MULLER, H. J., and S. M. GERSHENSON, 1935 Inert regions of the chromosomes as the temporary products of individual genes. Proc. nat. Acad. Sci. **21**: 69–75.

PANSHIN, I. B., 1935 New evidence for the position effect hypothesis. C. R. (Dokl.) Acad. Sci. U. R. S. S., N. S. **4**: 85–88.

1938 The cytogenetic nature of the position effect of the genes white (mottled) and cubitus interruptus. Biol. Zh., **7**: 837–868.

SCHULTZ, J., 1936 Variegation in Drosophila and the inert chromosome regions. Proc. nat. Acad. Sci. **22**: 27–33.

SCHULTZ, J., and T. CASPERSSON, 1939 Heterochromatic regions and the nucleic acid metabolism of the chromosomes. Arch. Zellforsch. **22**: 650–654.

SITKO, N., 1938a Mutation and aberration in inverted X chromosomes. Acad. Sci. Ukrainian S. S. R., Memoirs on Genetics **2**: 4–30.

1938b The dependence of the mutational process on chromosome reconstructions in translocations. Acad. Sci. Ukrainian S. S. R., Memoirs on Genetics **2**: 31–49.

STURTEVANT, A. H., 1925 The effects of unequal crossing-over at the Bar locus in Drosophila. Genetics **10**: 117–147.

SUCHE, M. L., D. R. PARKER, M. BISHOP, and A. B. GRIFFEN, 1939 Reversal of lethal factors. Genetics **24**: 88.

SUTTON, EILEEN, 1943 Bar eye in *Drosophila melanogaster*: A cytological analysis of some mutations and reverse mutations. Genetics **28**: 97–107.

TSUBINA, M. G., 1939 Comparative rate of appearance of translocations in a normal and aberrated chromosome-complex of *Drosophila melanogaster* Mgn. Trans. Zoo-Biol. Inst. Sai. Res. (Kharkov) **6**: 227–236.

WHITE, M. J. D., 1945 Animal cytology and evolution. viii+375 pp. Cambridge: Univ. Press.

ZELENY, C., 1919 A change in the bar gene of Drosophila involving further decrease in facet number and increase in dominance. J. gen. Physiol. **2**: 69–71.

INHERITANCE OF BETA-CAROTENE IN TOMATOES[1]

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BETA-carotene is the principal vitamin A active carotenoid found in tomato fruits. This pigment, however, constitutes only about 5 percent of the total carotenes present in commercial red-fruited varieties. Almost all of the remaining 95 percent of carotene is lycopene. In spite of the relatively small percentage of *beta*-carotene occurring in red-fruited varieties, tomatoes are classified, nevertheless, as a "good" source of vitamin A for the human diet (HEINZ 1942).

In 1942 a study aimed at increasing the vitamin content of tomato selections by breeding was initiated at this station. Progress toward the production of varieties genetically constituted to produce high concentrations of provitamin A and ascorbic acid has been reported (LINCOLN *et al.* 1943, 1949; KOHLER *et al.* 1947). It also has been reported that selections were made during the course of this work in which *beta*-carotene constituted 95 percent of the total carotenes (KOHLER *et al.* 1947). This paper reports the results of studies of the inheritance of *beta*-carotene in crosses between high lycopene (low *beta*-carotene) and high *beta*-carotene tomato selections.

PREVIOUS LITERATURE

The results of several studies on the inheritance of tomato flesh and skin color have been summarized by BOSWELL (1937). Most commercial varieties are red fleshed with yellow skin and therefore carry the dominant alleles *RTY* of yellow flesh (*r*), tangerine, orange flesh (*t*) and colorless skin (*y*). LE ROSEN *et al.* (1941) have shown that flesh and skin color are genetically and chemically unrelated. The *R* gene for red flesh color affects only the red and yellow plastid pigments, chiefly lycopene, but to a smaller extent other carotenes and xanthophylls, while the *Y* gene for yellow skin has no effect on plastid pigments but causes a ten-fold increase in an alkali soluble epidermal pigment. Recently LESLEY and LESLEY (1947) presented evidence that three or more genes determine the red-yellow color series in subgeneric crosses of Lycopersicon.

In the initial paper, LINCOLN *et al.* (1943) reported obtaining a selection containing 67.5 γ/ gm of *beta*-carotene or nine times as much as the content of the highest commercial variety. The same group, KOHLER *et al.*, (1947), later reported individual plant fruits containing as much as 115 γ/gm of *beta*-carotene (95 percent of total carotenes), while others of commercial fruit size contained

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83 γ/gm. LESLEY and LESLEY (1947) reported individual plants containing 75 and 90 percent of total fruit pigments as *beta*-carotene but did not give absolute quantities.

METHOD

Crosses to produce F_1 seed were made in the greenhouse. F_2 seed was obtained by selfing F_1 plants in the greenhouse. F_2 seed was obtained from open-pollinated flowers of F_2 plants grown in a field block.

Genetic populations were grown, sampled and the fruits were analyzed chemically as described in detail in a previous publication (ZSCHEILE and PORTER 1947). In brief, representative fruits were homogenized in a Waring Blendor, and a weighed aliquot was extracted with acetone and hexane (75:60). After filtration acetone was removed from the hexane by washing with H_2O. Carotenols and esters were then removed from the hexane with 90 percent methanol and 20 percent KOH in methanol. The hexane solution was washed three times with water and then brought to a volume of 100 ml. Spectroscopic readings (for *beta*-carotene and lycopene determinations) were made directly on the resultant hexane solution with a Beckman spectrophotometer. Check values for *beta*-carotene content were obtained by chromatographically separating this pigment from other carotenes on a MgO-Super Cel column. The *beta*-carotene solution was then analyzed spectroscopically.

RESULTS

The high *beta*-carotene parent (4079-5012-9-13) used in these studies was an F_6 single plant selection of the cross Indiana Baltimore $\times F_1$ (Rutgers $\times$ *L. hirsutum* P.I. 126445). The percentage of *beta*-carotene of 25 sib plants from which this plant was selected ranged from 93 to 98 percent of the total carotenes of the fruits. However, fruits of these plants varied from 54 to 115 γ/gm in *beta*-carotene content. Cuttings were made of the desired plant which had a fruit size of 35 grams and contained 115 γ/gm of *beta*-carotene which was 96 percent of the total carotenes present.

The low *beta*-carotene parent was a single plant from an Indiana Baltimore seed stock. It had a fruit size of 180 grams and a carotene content of 112 γ/gm, of which 6 percent was *beta*-carotene.

Cuttings of the two parental plants were rooted and grown to maturity in the greenhouse. F_1 seed was produced with the Indiana Baltimore selection as the seed parent. F_2 seed was obtained in the greenhouse from 8 F_1 plants. Two fruits from each plant were used as the seed stock from which the F_2 population was grown. Analyses were made of the parental, F_1 and F_2 populations grown in the field in 1946. The results of these analyses expressed as percentage of total carotenes present as *beta*-carotene are given in figure 1. Chromatographic analyses showed that lycopene and *beta*-carotene were the only carotenes present in quantities greater than a trace in these populations. The average percentage of *beta*-carotene in the high *beta*-carotene parent was 93, in the low *beta*-carotene parent, 10, and in the F_1, 61. This would indicate that the factor (or factors) responsible for the synthesis of *beta*-carotene is an incompletely dominant one. The mean percentage of *beta*-carotene in the entire F_2 popula-

tion was 55 percent, almost exactly the mean value of the two parents. There are three distinct peaks in the distribution curve of the 209 F_2 plants analyzed. These peaks occur at 12, 55, and 88 percent of carotenes as *beta*-carotene and coincide almost exactly with the mid-point for the distribution in Indiana Baltimore, the F_1 and the 4079 = 5012-9-13 population. The distribution of this curve fits the 1:2:1 ratio that would be expected if the genetic constitution of the two parents as regards factors for carotene synthesis differed by a single incompletely dominant gene. The distribution of observed and expected plants on the basis of 0–24.9 percent, 25–74.9 percent, and 75–100 percent *beta*-caro-

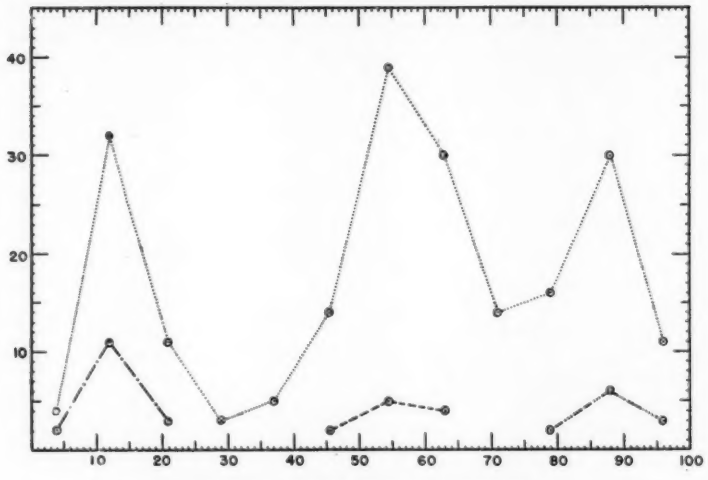

FIGURE 1. Distribution of results of analyses for percentage of *beta*-carotene for the parental, F_1 and F_2 populations, of a cross between high and low *beta*-carotene parents—F_6 Sel. 4079 = 5012-9 13 by Indiana Baltimore.

tene is given in table 1. The observed values closely approach that expected on a 1:2:1 hypothesis. The χ^2 value for these data is 0.79 .The simple correlation between fruit weight and total carotenes was 0.137, between fruit weight and lycopene, 0.047, and between total carotenes and lycopene, 0.105. The multiple R for these characters was 0.151. It is apparent that in this cross no significant correlation exists between these characters.

In 1947, 169 F_3 progenies of 9 plants each were grown from seed of open-pollinated fruits. The progenies were classified visually into 39 orange-fruited progenies, similar to the high *beta*-carotene parent, 92 intermediate or segregating progenies, and 38 red-fruited progenies. This classification included

in the parent-like groupings those progenies that had one plant classified as intermediate with all other plants of the parental class. This was considered reasonable inasmuch as open-pollinated F_3 seed was used. Unreported work has shown that although the average percentage of outcrossing may be only about 1 percent, individual fruits may have up to 100 percent of crossed seeds. The observations on the F_3 population, as classified, again fit the hypothesis of a monofactorial, incompletely dominant gene for *beta*-carotene production. These data had a χ^2 value of 1.34.

The above visual observations were checked with chemical analyses to determine the accuracy of visual classification. An 11 percent discrepancy was

TABLE 1

Number of observed and expected plants in an F_2 population with the designated percentage of beta-carotene and χ^2 value for the fit of these data to a 1:2:1 hypothesis.

PERCENT *beta*-CAROTENE	OBSERVED NUMBER	EXPECTED NUMBER	χ^2
0– 24.9	57	52.25	0.24
25– 74.9	105	104.50	0.02
75–100	47	52.25	0.53
Total	209	—	0.79

observed between the two classifications on 60 samples classified by both systems.

The gene present in the 4079-5012-9-13 stock, which is characterized by its production of *beta*-carotene, has been designated *B*. It is incompletely epistatic to *R*. It is believed *B* functions in the formation of *beta*-carotene from lycopene and therefore the *B* gene requires the *R* gene to express its effect. An alternative view would suggest that *B* is a member of an allelic series of genes for carotene formation. There is little evidence available at present to support this suggestion.

There is no evidence from the breeding program with either Rutgers or Indiana Baltimore as recurrent varieties that the increased *beta*-carotene content in the high *beta*-carotene selections is due to an increase in the total carotenes. Instead the increased *beta*-carotene content is developed at the expense of lycopene. A trend has been observed for total carotenes to decrease in selections in the breeding program in spite of some selection towards high total carotenes. This indicates some association between a high percentage of *beta*-carotene and a lowered total carotene content.

DISCUSSION

Tomatoes which contain principally *beta*-carotene differ from those which contain mostly lycopene by a single incompletely dominant gene, *B*. The inheritance of *beta*-carotene is similar to the inheritance of yellow and tangerine colors in tomatoes in that single factor differences exist between fruit of these

colors and red colored fruit, but the inheritance of *beta*-carotene differs in being incompletely epistatic to lycopene, whereas the factors for yellow and tangerine colors are recessive to lycopene. Thus, in crosses with high *beta*-carotene parents, F_2 plants with intermediate colored fruits, orange-red or red-orange, will be obtained as well as the parental red and orange types.

Tomato fruits bearing the factor for yellow color in the homozygous recessive state are almost lacking in carotenes (usually less than 5 micrograms per gram of fruit). Tomato plants homozygous recessive for tangerine fruit color yield fruits containing several pigments and colorless polyenes of which the major ones are *zeta*-carotene, prolycopene and phytofluene (LE ROSEN, *et al.* 1941, 1942; PORTER and ZSCHEILE 1946 a, b; MACKINNEY and JENKINS 1949). Since the factors for the formation of lycopene (*R*, *T*) are dominant to the factors for yellow, (*r*, *T*) and (*r*, *t*), and in part to tangerine (*R*, *t*) color in tomatoes, it can be suggested that lycopene is formed from simpler compounds, two of which are *zeta*-carotene and phytofluene. It is assumed carotenoid synthesis takes place in several steps and that a different gene is necessary to mediate each successive step. It is further assumed dominance means the reaction chain is carried at least one step further. The factor for *beta*-carotene synthesis in tomatoes is incompletely dominant to that for lycopene formation, but since the factors for lycopene formation are dominant to the factors for the pigments and colorless polyenes of yellow and tangerine tomatoes and also since lycopene is intermediate in structure to them and *beta*-carotene, it seems highly probable *beta*-carotene is formed from lycopene. If so, the following scheme would indicate its route of formation:

$$\text{Colorless precursor} \xrightarrow[\text{steps?}]{\text{No. of}} \text{Phytofluene} \rightarrow \textit{Zeta}\text{-Carotene} \xrightarrow{\text{genes } R,\ T} \text{Lycopene} \xrightarrow{\text{gene } B} \textit{Beta}\text{-Carotene.}$$

In considering the action of genes in the formation of carotenoids the action of enzymes must be considered. If one gene controls one enzyme, a deduction for which considerable evidence has been advanced, then the rate of formation of a compound (*beta*-carotene, lycopene) will be determined both by the quantity of enzyme and the conditions prevailing for its action. If in the heterozygous F_1 (*RRBb*) of a cross between *beta*-carotene and lycopene selections there are twice as many enzyme molecules for forming lycopene as *beta*-carotene then the *beta*-carotene content of the selection will be approximately equal to the lycopene content if *beta*-carotene is formed from lycopene and the efficiencies of the two enzymes are equal. This was actually found to be the case in the F_1 selections studied.

In preliminary crosses involving parents of greater genetic diversity than those used in this study it has been found that the inheritance of *beta*-carotene does not follow the expected pattern. For example, in a cross of a *L. pimpinellifolium* selection containing 200–350 micrograms of lycopene with the high *beta*-carotene selection used in this study it was expected that F_2 selections would be obtained containing at least 200 micrograms of *beta*-carotene. Such

selections were not obtained in the limited number of F_2 selections examined. Instead a strong linkage between factors for a high total carotene content and lycopene, and a low total carotene content and *beta*-carotene apparently exists in this particular cross. The same relationship has been observed in advanced generations of a cross between *L. esculentum* varieties and high *beta*-carotene selections. The *beta*-carotene content has declined slowly as homozygosity of the selections has been approached.

SUMMARY

The inheritance of *beta*-carotene in the cross of a low *beta*-carotene tomato variety with a high *beta*-carotene selection has been studied. A single gene, incompletely epistatic to *R*, is postulated for the inheritance of *beta*-carotene on the basis of analyses of F_1, F_2, and F_3 populations of the above cross. This gene is designated as *B*. The gene is considered to act on lycopene to form *beta*-carotene. Other genes (those dominant to yellow and tangerine) are assumed to convert simpler compounds including *zeta*-carotene and phytofluene to lycopene when present in the heterozygous or homozygous dominant state.

Extremely high *beta*-carotene selections were not obtained as expected in the F_2 generation from crosses of a *beta*-carotene parent with selections extremely high in lycopene content. A linkage of factors for high carotene content and a high lycopene content in certain crosses is suggested.

LITERATURE CITED

BOSWELL, V., 1937 Improvement and genetics of tomatoes, peppers, and egg plant. U. S. D. A. Yrbk. p. 176–206.

HEINZ, H. J. and Co., 1942 Nutritional charts, 11th ed.

KOHLER, GEO. W., R. E. LINCOLN, J. W. PORTER, F. P. ZSCHEILE, R. M. CALDWELL, R. H. HARPER, and W. SILVER, 1947 Selection and breeding for high *beta*-carotene content (provitamin A) in tomato. Bot. Gaz. **109**: 219–225.

LE ROSEN, A. L., F. W. WENT, and L. ZECHMEISTER, 1941 Relation between genes and carotenoids of the tomato. Proc. nat. Acad. Sci. **27**: 236–242.

LE ROSEN, A. L., and L. ZECHMEISTER, 1942 Prolycopene. J. Amer. chem. Soc. **64**: 1075–1079.

LESLEY, J. W., and M. M. LESLEY, 1947 Flesh color in hybrids of tomato. J. Hered. **38**: 245–251.

LINCOLN, R. E., G. W. KOHLER, W. SILVER, and J. W. PORTER, in press. Breeding for increased ascorbic acid content in tomatoes. Bot. Gaz.

LINCOLN, R. E., F. P. ZSCHEILE, J. W. PORTER, G. W. KOHLER, and R. M. CALDWELL, 1943 Provitamin A and vitamin C in the genus Lycopersicon. Bot. Gaz. **105**: 113–115.

MACKINNEY G., and J. A. JENKINS, 1949 Inheritance of carotenoid differences in *Lycopersicon esculentum* strains. Proc. nat. Acad. Sci. **35**: 284–291.

PORTER, J. W., and F. P. ZSCHEILE, 1946a Carotenes of Lycopersicon species and strains. Arch. Biochem. **10**: 537–545.

1946b Naturally occurring colorless polyenes. Arch. Biochem. **10**: 547–551.

ZSCHEILE, F. P., and J. W. PORTER, 1947 Analytical methods for carotenes of Lycopersicon species and strains. Industr. Engrg. Chem., Anal. Ed. **19**: 47–51.

HERITABILITY OF THRESHOLD CHARACTERS*[1]

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WITH AN APPENDIX BY ALAN ROBERTSON

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THE amount of selection pressure applied on the phenotypic level to metric characters can be expressed in terms of the selection differential, defined as the extent of superiority of the individuals which are the parents of the next generation (weighted for the relative number of offspring they produce) over the average of their own generation. For characters showing a discontinuous distribution the measurement of selection pressure can be expressed in terms of selection coefficients, that is, the relative number of offspring produced by each of the phenotypically classified categories of individuals in the population.

THE DEGREE OF HERITABILITY

The effective amount of selection pressure, however, in both cases depends on the degree to which the phenotypic variation, on which the selection differential and the selection coefficients are based, is reflected by genetic variation. It seems rather obvious that the changes in the genetic composition of a population from generation to generation, changes of which the shifts in the phenotypic mean will be representative (under a constant environment), are thus a function of the accuracy with which either nature or man recognizes genetic differences on the basis of phenotypic differences between individuals or groups of individuals.

Intimations of this fact were apparent to early geneticists. Many of them recognized that a phenotype represents a combination of genetic and environmental effects, of which only the first would contribute to changes in a population which are attributable to selection. As a single example the viewpoint of YULE (1906) may be cited. In discussing ancestor-offspring correlation, he stated: "A complete theory of heredity should take into account, besides germinal processes, the effect of the environment in modifying the soma obtained from any given type of germ-cell—an effect which is hardly likely to be negligible in the case of such a character as stature."

As early as 1910 WEINBERG (1909, 1910) suggested methods of separating genetic and environmental components of total phenotypic variability, but his contribution to the subject was overlooked for many years, sharing the fate

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of his independent discovery of what was subsequently referred to in genetic literature as HARDY's binomial (see STERN 1943).

It was only several years later that WRIGHT (1917 *et seq.*) and FISHER (1918), independently of each other and apparently unaware of WEINBERG's papers, developed comprehensive techniques of dealing with the problem. The first of WRIGHT's papers dealt with a case in which the genetic and the environmental types of variation were separated by experimental rather than statistical means. This is, of course, essentially the procedure used by JOHANNSEN (1903), who, however, did not attempt to assess the relative proportions of the two types of variation in a population.

WRIGHT's material consisted of inbred lines of guinea pigs. His analysis included not only the measurement of the relative amounts of genetic and environmental variation, but also the separation of genetic from environmental correlation between two traits by the use of the technique of covariance (at that time as yet an uncoined term).

A subsequent contribution of WRIGHT (1918) first presented his method of path coefficients, elaborated upon by him later (1921, 1934a), a method which permits the statistical separation of environmental and genetic variation in general populations. Simultaneously and independently FISHER (1918) dealt with the same subject, which he also elaborated upon later (FISHER, IMMER and TEDIN 1932). The developments finding their origin in the respective contributions of WRIGHT and FISHER appeared to proceed to a considerable degree independently of each other. Thus the statistical separation of genetic and environmental correlations between different traits was eventually described by SMITH (1936) basing himself on FISHER's work, and by HAZEL (1943), whose technique was derived from the method of path coefficients combined with the analysis of covariance.

The fraction of the total phenotypic variance due to genetic differences came to be known as the degree of heritability or simply as heritability (LUSH 1945) usually symbolized by h^2. It is, however, necessary to distinguish between two different statistics which may be understood by this term, namely the *total* and the *additive* h^2. The distinction between them may be made clear by reference to *genotypic* and to *additive genetic* values of individuals.

The former (with respect to whatever trait is considered) is defined as the mean of an individual's genetic replicates exposed to the total array of environments. The concept of the additively genetic value is based on the theoretical possibility of assigning particular values to each gene contained in the population. These can be so chosen that the variance of the differences between their sums for each individual (the additive genetic value) and the genotypic values for the same individuals is at a minimum. If the genotypic value of every individual is the same as its additive genetic value, genetic variance is completely additive. If this is not true, the additive portion of the genetic variance is equal to the variance of the additively genetic values; the amount by which the total genotypic variance exceeds this value is the non-additive genetic variance.

The additive heritability then refers to the ratio of the additively genetic

to the total variance. In the absence of non-additive genetic variation, and when there is no correlation between genotypic level of individuals in the population and its environmental variance,—(1) the correlation between the genotypic and phenotypic values is equal to the square root of the heritability, (2) the regression of genotypic value on phenotypic value is linear, and (3) the expectation of phenotypic value of offspring is equal to the mean genotypic value of the parents (excluding deviations from this rule due to sex linkage). It follows that genetic gain from mass selection is equal to the product of the heritability and the mean phenotypic advantage of the parents over the mean of their generation (the selection differential). A combination of these propositions with a knowledge of the genetic and non-genetic correlations between members of a family leads to simple formulas for computing gains from family selection and from combined family and individual selection on the basis of an index, as well as formulas for computing optimum indexes for this purpose and for simultaneous selection with respect to more than one character at the same time (HAZEL 1943; LUSH 1947).

When some non-additive genetic variance is present, the expectation of the phenotypic value of the offspring is not necessarily equal to the mean genotypic value of the parents. A tendency for the regression of offspring towards the mean of the parental generation is present, so that selection is less effective than it might be expected to be on the basis of the genotypic values of the parents. Furthermore, such gains as are obtained in one generation of selection will not necessarily persist in subsequent generations (LUSH 1945).

The removal of the second restriction noted (independence of the average genotypic value from the environmental variance) may cause the regression of genotype on phenotype to be non-linear. In this situation, the proportion of the phenotypic selection differential which will be realized as a genetic gain will depend on selection intensity and other factors. This will generally lead to a positive or negative bias of *estimate of gain* based on the conditions of strict additiveness.

HERITABILITY OF ALL-OR-NONE CHARACTERS

Most of the applications of the concept of heritability have until recently been made with respect to characters showing a continuous distribution on the phenotypic scale. The importance of this statistic, however, extends equally to traits which, while possibly based on a continuous or normal distribution of genotypic values, may be expressed phenotypically on an all-or-none, or generally speaking discontinuous basis.

It was once more WRIGHT (1920) who first dealt with the general subject. Since the average phenotype of groups of related individuals for such traits can be expressed in terms of percentage incidence, he evolved a transformation of percentage data to a normalized form. The transformation was described by him in detail (1926) under the name of "inverse probability." Essentially the same method was later proposed by BLISS (1935) as the "probit" transformation. It is the latter term which has gained currency in the literature and

under which the statistical properties of transformed distributions were described (for instance by FINNEY 1947).

By the use of his transformation WRIGHT was able to determine the relative amounts of genetic and environmental variation in such traits as digit number in guinea pigs (1934b, 1934c) and flower color in Linanthus (1943). Subsequently LUSH, LAMOREUX and HAZEL (1948) introduced the subject into the realm of animal breeding by considering the heritability of viability in the fowl, a matter which also received attention from ROBERTSON and LERNER (1949).

In general, it seems reasonable to treat characters with an all-or-none phenotypic expression as dependent on one or several more or less continuously variable underlying variates. The value of each underlying variate in a particular individual, according to this view, would depend both on genetic and non-genetic factors, and the appearance of the character would require that some threshold be exceeded. The genotype thus determines a probability of the character appearing, or the proportion of environments in which it will actually come to expression.

This viewpoint permits the determination of heritability of a character measured in terms of its probability of appearance, with values of 0 and 1 assigned to the alternative phenotypic expressions. This scale of measurement will be referred to henceforth as the *p scale*. Heritabilities determined from it may provide a basis for computation of genetic gains expected from selection and for construction of selection indexes. However, in some instances, serious inaccuracies may result, since genetic variance which may be completely additive for the underlying variate may lose this property on the p scale. Since the limiting genotypic values are 0 and 1, it is highly unlikely that a given gene substitution would have the same effect near these limits as in the middle of the range.

Similarly the environmental variance of the underlying variate may be independent of the mean genotypic value (the level of incidence or p), but this property may be lost on the p scale. This is apparent from the fact that environmental variance on the p scale, which, of course, is the total variance for any given fixed genotype, is equal to pq, where $q=1-p$. For the range of genotypes with p values from, say, .35 to .65, the environmental variance is reasonably constant, but in the extreme ranges of p from 0 to 0.1 and from 0.9 to 1.0, it is nearly directly proportional to p and to q respectively. Finally, the categorical nature of the p scale may obscure the finer degrees of measurable variation (as for example age of death, where viability in a set period of time is considered). All of these circumstances vitiate to some extent the utility of the degree of heritability determined on the p scale.

LUSH, LAMOREUX and HAZEL (1948) held that probit transformation avoids some of the objections to the p scale. As in the previously noted work of WRIGHT, the transformation is based on the concept of an underlying variate with a normal environmental distribution, whose variance is independent of the genotypic level. The heritability on the probit scale is independent of the threshold value, above which the character will be manifested in individuals whose underlying variate exceeds it, whereas on the p scale heritability (pro-

viding the genetic variance is not too large compared to the environmental variance) varies approximately in proportion to $\bar{z}^2/(\bar{p}\bar{q})$, where $\bar{z}$ is the ordinate of a unit standard normal curve cutting off an area equal to $\bar{p}$. The p scale heritability then, in terms of the probability of an individual exhibiting the character, would be low for values of $\bar{p}$ near zero or unity and relatively high for intermediate values. ROBERTSON and LERNER (1949) have shown that the situation is similar where there are a number of underlying variates, although the distribution of h^2 is, of course, somewhat different in form.

The probit transformation may be a satisfactory one for the purpose of comparing heritability values but it is not apparent how it can be used for devising optimum selection indexes nor is it at all convenient in many situations for computing expected rates of gain. It appears useful therefore to investigate the conditions, if any, under which serious errors are likely to result from the use of the convenient p scale and the nature and magnitude of such errors. Such an investigation might disclose that the p scale can be used without hesitation in many situations and with some reservations or with corrections in others. The results of such a study might also suggest in a general way the degree to which a scale can deviate from the optimum one without leading to selection indexes that depart seriously from the optimum or to computations of gain that are much in error. Where calculations of the gain from mass selection based on the p scale lead to correct results there is good reason to conclude that calculations of the gain from family and combined selection will also be accurate, as well as that the usual methods for the computation of optimum indexes will also be applicable to a high degree of approximation. The converse of this statement is, however, not necessarily valid. The present investigation is chiefly concerned with a study of the gains that would result from mass selection on an all-or-none basis in comparison to estimates of gains whose computations are based on heritability determinations on the p scale, as well as with gains that would result could selection be based on direct observation of the underlying variate. The bearing of these findings on indexes for combined individual and family selection is discussed briefly.

THE MATHEMATICAL MODEL

The first step in the proposed investigation involves the comparison of genetic gains computed on the basis of h^2 determined from the p scale with those expected on the postulate of a normally distributed underlying variate. The assumptions involved are that:

1. There is an underlying variate whose value is the sum of a normally distributed environmental component and an independent normally distributed genetic component.
2. The character is present in all those individuals, and only those, in which the underlying variate exceeds a certain threshold value.
3. Gene substitutions have individually small and strictly additive effects on the underlying variate.

There is no claim made here that these conditions actually describe the

situation with respect to all characters of an all-or-none nature. It is, however, reasonable to believe that a model in which additiveness is the property of the underlying variate (on what will henceforth be referred to as the *x scale*) approaches actual situations more closely than one based on additive gene action on the p scale.

Figure 1 illustrates the model postulated. The solid-line curve (b) is a normal curve of unit area representing the distribution of the underlying vari-

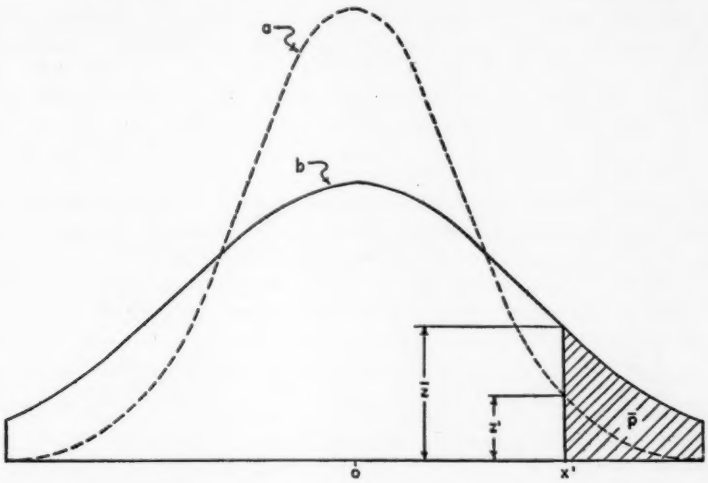

FIGURE 1. The model investigated. The solid line curve *b* represents the distribution of all individuals in the population. The abscissae (*x*) are values of the underlying variate expressed in standard deviation units. If this variate in any individual exceeds the threshold value x′, the character is expressed and p̄ is the proportion of all individuals in the population exhibiting the character. The dotted curve *a*, having the environmental standard deviation $\sqrt{1-\sigma G_x^2}$, represents the distribution of *x* for all individuals possessing the mean genotype of zero on the *x* scale. If this curve is shifted to the right or left so that its mean is not zero, it then represents the distribution of *x* for all individuals whose genotype on the *x* scale is the new mean. The area to the right of x′ under any such curve is the corresponding genotype on the p scale.

ate. It is convenient to take the standard deviation of this distribution as the unit of measurement on the x scale. The dotted curve (a) is a normal curve of unit area representing the distribution of all those individuals that possess the *mean* genotypic value; this distribution is therefore the environmental distribution and its variance is $(1-h_x^2)$ where h_x^2 is the heritability of the underlying variate and is also equal to the genetic variance on the x scale. The ordinate drawn to the right of the mean is the threshold value, all individuals to the right of this ordinate exhibiting the character.

The abscissa corresponding to the ordinate cutting off a tail of area p̄ where p̄ is the incidence of the character in the population is represented by x′. Its value can be obtained from tables of the normal distribution. The height

of the ordinate ($\bar{z}$) can also be similarly obtained. Since the dotted curve has unit area and a standard deviation of $\sqrt{1-h_x^2}$, the area under this curve to the right of the threshold is that of a unit standard normal distribution lying to the right of abscissa $x'/\sqrt{1-h_x^2}$. This area is the proportion of all those individuals possessing the *mean genotype* that exhibit the character. The proportion of all those individuals possessing *some other fixed genotypic value* that exhibit the character can similarly be obtained if one first subtracts from this abscissa the product of the factor $1/\sqrt{1-h_x^2}$ and the difference between the genotypic value in question (on the x scale) and the mean genotypic value. Genetic differences are thus thought of as shifting the mean of the environmental distribution, the threshold remaining fixed. Alternatively, they can equally well be thought of as shifting the threshold abscissa, the mean of the environmental distribution remaining fixed. Using either concept the genotype of an individual on the p scale is the proportion of environments in which it would exhibit the character.

For every genotypic value on the x scale there is a corresponding value (that is, proportion of individuals exhibiting the character) on the p scale. The determination of p values corresponding to given x values is easily accomplished by the method outlined above. Some idea of the degree of disparity of the two scales can be obtained by marking off a linear scale in x units and marking the corresponding points on a parallel scale in p units. Figure 2

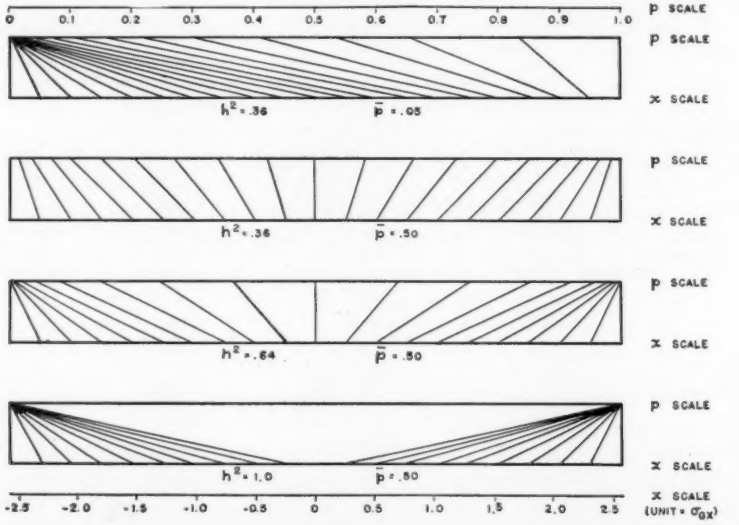

FIGURE 2. Comparison of x and p scales. The slanting lines in each example connect genotypes of the x scale (lower), on which gene differences are postulated to have additive effects, with corresponding genotypes of the probability or p scale (upper). The variability of the spacing of the intersects on the p scale is a measure of the inequality of the effects of gene substitutions expressed on this scale at different genotypic levels.

shows some scale comparisons of this sort for heritabilities of 0.36, 0.64 and 1.0 and for incidences of 0.05 and 0.5. The range shown is ± 2.58 standard deviations of the genetic distribution each side of the mean so that 99 percent of genotypes are included. The distribution of p values for the case in which the underlying variate has a heritability of unity is, as shown, discontinuous. Inspection of these scale comparisons indicates but little disparity where heritability is low and the incidence close to 0.5 but may lead to some doubt as to the applicability of the usual estimation of gains from selection on the basis of heritability determinations where h_x^2 is high or the incidence is near zero or unity.

EMPIRICAL DETERMINATION OF ADDITIVE HERITABILITY ON THE P SCALE

Before proceeding with the discussion of the relationship between heritabilities on the x and p scales it may be worth while to present the method of heritability estimation on the p scale as outlined by ROBERTSON and LERNER (1949) based on actual data, which may also serve to illustrate the relationship between the heritability on the p scale and that on the x scale.

The data used for the present purpose are derived from the material reported on by LERNER and CRUDEN (1948). They computed the heritability of egg production for various periods of time of birds surviving the first laying year in a flock of Single Comb White Leghorns. The roughly normal underlying variate considered here is the total first year egg production (from beginning of lay till September 30th of the second year of life) of the same birds. Thus the heritability estimates computed by the conventional methods represent in our notation h_x^2.

The estimates presented by LERNER AND CRUDEN were based on two separate series of birds hatched in consecutive years. Each series in turn contained two sub-flocks (lines), one selected primarily for egg production, and

TABLE 1

The material used in the analysis (derived from LERNER *and* CRUDEN *1948).*

SERIES	LINE	NUMBER OF SIRES	NUMBER OF DAMS	NUMBER OF DAUGHTERS	AVERAGE GENETIC RELATIONSHIP WITHIN FAMILY	h_x^2 ESTIMATE BY LERNER AND CRUDEN
S	Production	6	22	111	.314	−.034
	Others	11	50	235	.297	.587
	Total	17	72	346	.302	.381
T	Production	10	47	226	.296	.272
	Others	11	46	225	.305	.423
	Total	21	93	451	.301	.358
S+T	Total	38	165	797	.301	.369

the other for various other traits listed in their report. The distribution of birds by sub-flocks and series is shown in table 1.

Several methods of h_x^2 computation were employed by LERNER and CRUDEN. The estimate shown in table 1 is the one based on information from the correlations between both full sisters and half sisters. For our purposes the values of h_x^2 thus reported need some adjustment, because the p scale analyses to be presented were conducted by partitioning the total variance into that between and within sire families. Each sire family was therefore a mixture of full and half sisters. Hence, the genetic relationship within each family was greater than 0.25 (the value for half sisters) and less than 0.5 (the value for full sisters). The average genetic relationship was computed and is also given in table 1. Having obtained this constant the heritability values reported by LERNER and CRUDEN were recomputed on the basis of the usual statistical design.

The raw data were arranged for the p scale analyses in order of magnitude of individual egg production records for each sub-population separately. For combined sub-populations (by series) the data were rearranged in a similar fashion and again for the total group of birds. Nine levels of incidence were analyzed, from 0.1 to 0.9 ranging in intervals of 0.1. Thus for the lowest level the top ten percent of each sample considered were given values of 1 and the rest of the sample, values of 0. For the second level the top 20 percent were assigned values of 1 and the rest values of zero, and so forth.

The formulas derived or cited by ROBERTSON and LERNER (1949) were then applied to the data. The estimates of heritability obtained by this method include a certain amount of non-additive variance and will be designated as h_p^2, as contrasted to h_{pa}^2 for strictly additive and h_{pt}^2 for the total heritability (see following section). By way of recapitulation the essential expressions used were the following ones:

$$h_p^2 = \frac{\chi^2 - (N - 1)}{r n_0},$$

where

$$n_0 = \sum n - \frac{\sum n^2}{\sum n} - (N - 1),$$

while N = number of sires,
n = number of daughters per sire,
r = the genetic relationship between the members of a sire family.
and χ^2 = the heterogeneity Chi-squared in the 2×N table.
The standard error of estimate of h_p^2 is σ_t/r,
where

$$\sigma_t = \frac{[1 + (n - 1)t](1 - t)\sqrt{2}}{\sqrt{n(n - 1)(N - 2)}},$$

and t = the phenotypic correlation between the members of the sire family and equals rh_p^2.

The h_p^2 values may be compared with the h_{pa}^2 transformations from h_x^2 by the method described in the following section ($h_{pa}^2 = \bar{z}^2 h_x^2 / \bar{p}\bar{q}$), which appear in the last line of table 2. It may be seen that in general $h_p^2 > h_{pa}^2$ as

TABLE 2

Heritability estimates.

SERIES	LINE	h_x^2 FROM TABLE 1	RECOMPUTED h_x^2	ESTIMATES FROM BINOMIAL DATA FOR GIVEN LEVEL								
				.1	.2	.3	.4	.5	.6	.7	.8	.9
S	Production	−.034	.030	−.14	−.09	−.11	−.03	−.08	−.10	−.10	.18	.17
	Others	.587	.530	.18	.37	.42	.50	.45	.47	.45	.27	.19
	Total	.381	.358	.08	.23	.26	.33	.28	.29	.28	.24	.18
T	Production	.272	.236	0	−.05	.06	.09	.06	.10	.11	.17	.07
	Others	.423	.472	.22	.35	.55	.49	.33	.31	.22	.23	.35
	Total	.358	.364	.11	.16	.31	.30	.20	.20	.17	.20	.21
S+T	Total	.369	.362	.10	.19	.29	.31	.23	.24	.26	.22	.20
	Standard Error of Estimate			.06	.08	.10	.10	.08	.09	.09	.08	.08
	h_{pa}^2 computed from x scale			.12	.18	.21	.22	.23	.22	.21	.18	.12

would be expected from the fact that some non-additive variance is included in the first. By and large, however, the agreement between h_p^2 and h_{pa}^2 is in the light of the standard errors of estimate reasonably good.

COMPARISON OF HERITABILITIES ON THE P AND THE X SCALES

There is no distinction between additive and total heritability on the underlying or x scale because, in accordance with the postulates listed, gene differences have strictly additive effects on this scale. On the p scale, however, some of the genetic variance is non-additive and the non-additive portion may be very large where heritability on the x scale is very high or the incidence close to 0 or 1.0. It is therefore desirable for the purposes of the present investigation to compute both the additive heritability (additive portion of genetic variance divided by total variance) and the total heritability (total genetic variance divided by total variance).

For the special case in which $h_x^2 = 1.0$, expressions giving the total and the additive heritabilities on the p scale are easily obtained. Here, all of the variance on the p scale is genetic and is equal to $\bar{p}\bar{q}$. Multiplication of this value by the square of the correlation (r_{xp}^2) between p and x will yield the additively genetic variance. This correlation is the covariance divided by the square root of the product of the variances of p and x; since p equals zero for values of x less than x′ and equals unity for values of x greater than x′, the covariance can be expressed as follows:

$$\frac{\int_{-\infty}^{+\infty} (0)yxdx + \int_{x'}^{+\infty} (1)yxdx}{\int_{-\infty}^{+\infty} ydx} - \bar{x}\bar{p}.$$

The first term in the numerator is zero as is also the product $\bar{x}\bar{p}$ because the mean value of x is zero. The denominator of the fraction is equal to unity. The remaining integral, and hence the covariance, is simply

$$\left[-\frac{1}{\sqrt{2\pi}}e^{-x^2/2}\right]_{x'}^{\infty} = \bar{z}.$$

The variance of x is unity and that of p is $\bar{p}\bar{q}$, so the expression for r_{xp} becomes $\bar{z}/\sqrt{\bar{p}\bar{q}}$. The product of the square of this correlation and the total genetic variance ($\bar{p}\bar{q}$), is equal to $(\bar{z}^2/\bar{p}\bar{q})\bar{p}\bar{q}$ or to $\bar{z}^2$, which is thus the additively genetic variance. The additive heritability on the p scale (h_{ps}^2) is then for this case equal to $\bar{z}^2/\bar{p}\bar{q}$.

This expression in a more general form,

$$h_{ps}^2 = \bar{z}^2 h_x^2/\bar{p}\bar{q},$$

can be used irrespective of the value of h_x^2. The rationale behind the deduction can be briefed as follows. Were the genotypic values on the two scales linearly related, the genetic variance on the p scale would be equal to that on the x scale multiplied by the constant term $(dp/dx)^2$. The relation is not linear and dp/dx is not constant, but the use of the mean value of dp/dx will yield the additive genetic variance.[2] The height of the ordinate at x′, which for a population of individuals all possessing the mean genotype is z′ (fig. 1), is the value of dp/dx. The weighted mean value of z′ for all genotypes in the population is also the value of z which corresponds to $\bar{p}$ and is indicated by $\bar{z}$. The additive genetic variance on the p scale ($\sigma_{G_{ps}}^2$) is then: $\sigma_{G_x}^2\bar{z}^2 = \bar{z}^2 h_x^2$. The additive heritability on the p scale is this value divided by the total variance which, in a dichotomous distribution, is $\bar{p}\bar{q}$. We have then, $h_{ps}^2 = h_x^2\bar{z}^2/\bar{p}\bar{q}$.

The total heritability of character incidence on the p scale (h_{pt}^2) may be approximated in the following manner. The genetic distribution in terms of the x scale, which has been postulated to be normal in form and has a variance of h_x^2, is divided into a number of segments by ordinates spaced along the x axis. The genotype on the p scale of every individual within a segment is considered, as an approximation, to correspond to the mean genotype on the x scale of all individuals represented by this segment. The genetic variance of these p values, each weighted in proportion to the area of the corresponding segment, is then an approximation to total genetic variance. The quotient of this value by $\bar{p}\bar{q}$, the total variance, is an approximation to total heritability on the p scale. This heritability calculation can be carried to any desired degree of accuracy by choosing an appropriate number of segments. The results obtained by this method (the "segmental" method of table 3) in the present study are based on a division of the genetic distribution into 20 equal segments and a further division of the end segments into five equal sub-segments each, making 28 segments in all.

[2] See Appendix for a demonstration that the use of this mean value yields an exact expression for the additive genetic variance.

TABLE 3

*Heritabilities and genetic variances on the x and p scales.**

h_x^2	VARIATE	METHOD OF CALCULATION	INCIDENCE .05 .95	.1 .9	.2 .8	.3 .7	.4 .6	.5
0–1	h_{pa}^2/h_x^2	$z^2/\bar{p}\bar{q}$	.224	.342	.490	.576	.622	.637
.36	$\sigma_{G_{pt}}^2$	Segmental —	.00583	.0145	.0320	.0463	.0555	.0586
.36	$\sigma_{G_{pa}}^2$	$(\sigma_{G_{pt}}^2)(r_{xp}^2)$—segmental	.00378	.0111	.0282	.0436	.0538	.0574
.36	$\sigma_{G_{pa}}^2$	$z^2h_x^2$ —	.00383	.0111	.0282	.0435	.0537	.0573
.36	$\sigma_{G_{pe}}^2/\sigma_{G_{pt}}^2$	$1\text{-}r_{xp}^2$ —segmental	.353	.236	.120	.0600	.0294	.0205
.36	h_{pt}^2	$(\sigma_{G_{pt}}^2)(r_{xp}^2)$—segmental	.123	.161	.200	.221	.231	.235
.36	h_{pa}^2	$z^2h_x^2/(\bar{p}\bar{q})$ —	.0806	.123	.176	.207	.224	.229
.64	$\sigma_{G_{pt}}^2$	Segmental —	.0146	.0319	—	.0896	—	.111
.64	$\sigma_{G_{pa}}^2$	$(\sigma_{G_{pt}}^2)(r_{xp}^2)$—segmental	.00677	.0197	—	.0776	—	.102
.64	$\sigma_{G_{pa}}^2$	$z^2\,h_x^2$ —	.00681	.0197	.0502	.0774	.0955	.102
.64	$\sigma_{G_{pe}}^2/\sigma_{G_{pt}}^2$	$1\text{-}r_{xp}^2$ —segmental	.535	.383	—	.134	—	.0760
.64	h_{pt}^2	$\sigma_{G_{pt}}^2/(\bar{p}\bar{q})$ —segmental	.307	.355	—	.427	—	.442
.64	h_{pa}^2	$z^2\,h_x^2/(\bar{p}\bar{q})$ —	.143	.219	.314	.368	.398	.407
1.0	$\sigma_{G_{pt}}^2$	$\bar{p}\bar{q}$ —	.0475	.09	.16	.21	.24	.25
1.0	$\sigma_{G_{pa}}^2$	z^2 —	.0106	.0308	.0784	.1209	.1493	.1592
1.0	$\sigma_{G_{pe}}^2/\sigma_{G_{pt}}^2$	$1\text{-}z^2/(\bar{p}\bar{q})$ —	.7761	.6578	.5101	.4243	.3781	.3634

* Empty cells represent non-essential values.

The segmental method can be extended so as to provide a technique of computing the proportions of the total variance that are additive and non-additive, and hence also of a determination of h_{pa}^2 alternative to the exact one described above. The alternate determination provides a check on the degree of approximation involved in the segmental procedures. The proportion of the total genetic variance that is additive is equal to the square of the correlation between the additively genetic values and the genotypic values on the p scale of the individuals in the population (r_{xp}^2). This correlation is the same (under the condition that all gene substitutions have small individual effects) as that between corresponding genotypic values on the x and p scales because, under the condition mentioned, the effects of all possible gene substitutions for any given genetic background, and hence also the mean effect on the p scale for all genetic backgrounds, will be in direct proportion to the effects on the x scale. An approximation to this latter correlation can be obtained by dividing the covariance of the genetic values on the p and x scales of the segments of the genetic distribution by the square root of the product of the variances of these values. The product of the square of this correlation and the total genetic variance is then an approximation to the additively genetic variance,

$$\sigma_{G_{pa}}^2 = \sigma_{G_{pt}}^2 r_{xp}^2,$$

while the remainder of $\sigma_{G_{pt}}^2$ is the non-additive variance. The latter, on the assumption that individual gene differences have small effects, is epistatic and may be indicated by $\sigma_{G_{pe}}^2$. We may, then, write:

$$\sigma_{G_{pe}}^2/\sigma_{G_{pt}}^2 = 1 - r_{xp}^2.$$

This method of estimating epistatic variance is probably more accurate than that based on the difference between the approximately determined total variance and the exactly computed additive variance.

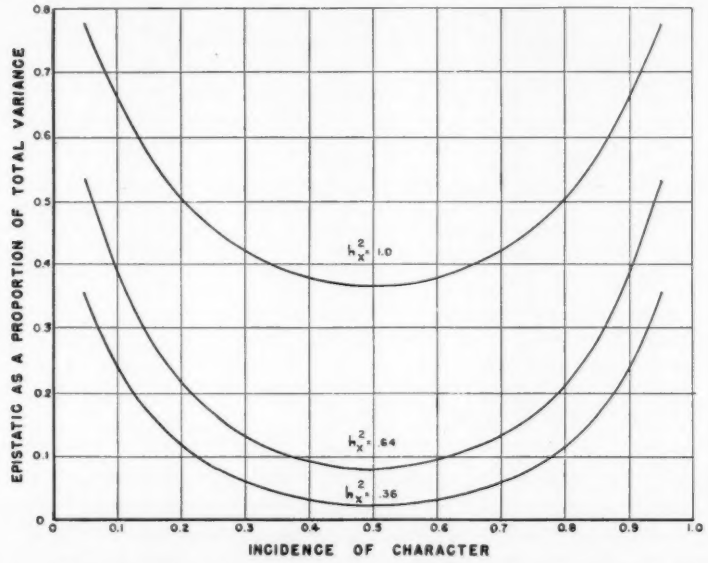

FIGURE 3. The proportion of total genetic variance that is epistatic, expressed as a function of character incidence in the population.

Table 3 presents the p scale estimates of variances and heritabilities obtained by the methods described for heritabilities of 0.36, 0.64, and 1.0 at varying levels of incidence. Very close agreement between the two sets of values of $\sigma_{G_{pa}}^2$ (segmental versus the exact) may be noted for h_x^2 of 0.36 and 0.64. Approximate methods are not required where h_x^2 approaches zero or unity.

Ratios of epistatic to total genetic variance on the p scale are also listed in table 3 and shown graphically in figure 3. It may be noted that the epistatic variance is trivial where the heritability is low and the incidence near 0.5 but may constitute most of the genetic variance under other conditions, having a value of over 77 percent of the total variance under the most extreme condition listed. It will be shown later that serious errors resulting from the use of heritability on the p scale in estimating gains from mass selection occur

only when epistatic variance forms a considerable proportion of the total genetic variance and when selection intensity is relatively high.

THE PROPORTION OF EPISTATIC VARIANCE INCLUDED IN HERITABILITY ESTIMATES

Intraclass correlations between full and half sibs are commonly used to estimate the degree of heritability of a trait (that is, the h_x^2 values in table 1). Since non-additive genetic variance does not contribute as much to such correlations as additive genetic variance does, such estimates will tend to be of a magnitude intermediate between additive and total heritability. The classic investigation of the contribution made by non-additive genetic variance to the correlation between relatives was carried out by WRIGHT (1935). In the model studied by him the values of a secondary character depend on the deviations of a primary character from an optimum. Here (as in our model) the genetic variance of the secondary character is partly epistatic even when that of the primary is completely additive. WRIGHT found, for the case of many genes, exclusively epistatic genetic variance, and no environmental variance, that the correlation between full sibs is one quarter; the value under the same conditions for half sibs is one sixteenth. These values are to be multiplied by the square of the heritability (h^4) of the primary character where environmental variance is present. However heritability on the secondary scale, all non-additive (epistatic) under the conditions mentioned, is the square of heritability on the primary scale, or h^4. Thus the correlation between full sibs on the secondary scale is one quarter of the heritability on the same scale; similarly the correlation between half sibs on the secondary scale is one sixteenth of the same value. If therefore the correlation between full sibs is doubled to obtain a heritability estimate (as is appropriate in the case of additive genetic variance) the result is only half the actual heritability. Likewise quadrupling the half-sib correlation yields one quarter the actual heritability. Finally, the variance components due to different dams mated to the same sire yields a heritability estimate including the proportion $4[(1/4)—(1/16)]$ or three quarters of the genetic variance.

For the model considered here, the fraction of the non-additive variance included in the estimates of heritability can be readily determined from a comparison of the previously calculated values for additive and total heritability with the estimates obtained from the three variance components noted in the previous paragraph. The total variance is equal to $\bar{p}\bar{q}$. If the p scale variance within full sib families is subtracted from this total, the variance component due to different sires and dams (intraclass full sib correlation) is obtained. Similarly the difference between the total variance and that within half sib families yields the variance component due to different sires (intraclass half sib correlation). Finally the difference between the two components thus obtained is the component due to different dams mated to the same sire.

The problem then calls for the determination of intra-family variances on the p scale. For full sibs in a population under random mating and in equilibrium the variance of family means on the x scale equals one-half of the genetic variance ($\sigma_G^2/2$). If the mean abscissae of segments of a unit normal

curve are multiplied by the square root of this variance, the true mean genotypes on the x scale of a representative array of families are obtained. The difference between such a mean value of any family and the threshold abscissa for the whole population is the effective threshold, in terms of total standard deviation units, for the family in question. However, the total variance within the family is only $(\sigma_G^2/2)+\sigma_E^2$ (where σ_E^2 represents environmental variance). Hence in order to obtain the equivalent threshold abscissa for a standard unit normal curve, the computed threshold abscissa for the family must be divided by $\sqrt{(\sigma_G^2/2)+\sigma_E^2}$. The area of the normal curve (p) corresponding to this standardized abscissa is, of course, the incidence of the character in the particular family, while $p(1-p)$ is the total variance within the family. Such variances can be computed for the whole array of families and their mean (weighted for the relative frequency of each kind of family in the population) is the total variance within full sib families. The analogous value for half sib families is obtained in a similar fashion. There the variance of family means on the x scale is $\sigma_G^2/4$, and the variance within families $(3/4)\sigma_G^2+\sigma_E^2$.

It should be noted that when the differences between the total and the

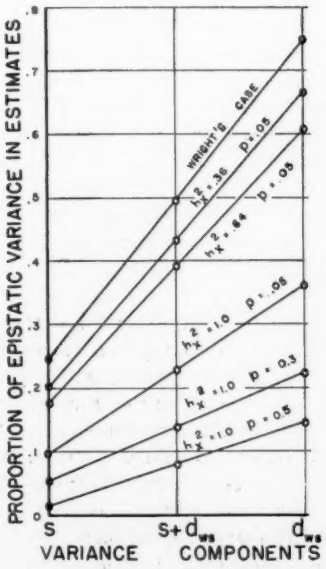

FIGURE 4. The proportion of the epistatic variance inculded in the numerator of heritability estimates based on variance components. Here s represents the component due to different sires, $s+d_{ws}$ represents the component due to the combined effect of different sires and dams, and d_{ss} represents the component due to different dams mated to the same sire.

within family variances are small, slight errors in the determination of the latter are considerably magnified in the final results. Some gain in accuracy can be achieved by computing directly the variance of the incidences in the array of families about their own mean, a method identical to the one described if the product of the mean incidence of the array of families by its complement is used as the total variance. This mean incidence is slightly different from the mean incidence in the population because of the approximation involved in taking the means of each segment as representative of all families within the segment. The modified method is sufficiently accurate for our purposes where the epistatic variance is not too small a proportion of the total variance. The calculations have been made, therefore, for heritabilities on the x scale of 0.36 and 0.64 with a population incidence of 0.05, and for an $h_x^2 = 1.0$ with population incidences of 0.05, 0.3 and 0.5. For the last three cases the determination of the proportion of epistatic variance included in the three kinds of estimates is probably correct within one to three parts in a hundred; for the first two the errors may be as high as one part in ten or twenty.

Figure 4 shows the proportions of epistatic variance, calculated in the manner just described, for the three types of heritability estimates considered. The proportions for WRIGHT'S model are shown for comparison. Although the proportions for the latter model do not depend on heritability, those on the p scale increase with decreased heritability, at least where the incidence is near zero or unity. The possibility is suggested that the maximum proportions may equal the corresponding ones for WRIGHT'S model. The components due to differences between sires by and large contain but little epistatic variance; those due to differences between dams mated to a sire include considerably more of it. In general it appears that in this model heritability estimates, based on the methods discussed, are likely to lie closer to additive heritabilities than to total heritabilities. Exceptions occur where the epistatic component is small and hence relatively less important.

THE COMPARISON OF CALCULATED AND ACTUAL GAINS FROM MASS SELECTION

In mass selection the estimated gain based on heritability on the p scale is simply the product of this heritability and the difference between the proportion of parental individuals exhibiting the character and the incidence in the unselected parental generation. Calculations have been made, based on the selection as parents of all individuals exhibiting the character, for h_x^2 of 0.36, 0.64, and 1.00 and for a range of incidences in the parental generation from 0.05 to 0.95. For each case two heritabilities on the p scale have been used, the additive heritability and the additive plus one quarter of the epistatic heritability. In view of the results shown in figure 4, most cases would be expected to fall within these limits. For comparison with these values, the actual gains that would be achieved have been calculated on the following basis.

The expected mean phenotypic value of the offspring of a group of parents

is equal to the parents' mean genetic values on the x scale, provided there is no correlation between the genetic values of the parents and the numbers of offspring they produce. In terms of the p scale the genetic gain from mass selection may be defined as the increase in the expected incidence of the offspring over the incidence in the parental generation (the selected parents being mated at random). An assumption to be made here is that the genetic variance on the x scale of such offspring is equal to that of the parental generation.

The mean abscissa of individuals possessing the character in a population with an incidence of p_p is equal to $\bar{z}/p_p$. Should all such individuals (or a ran-

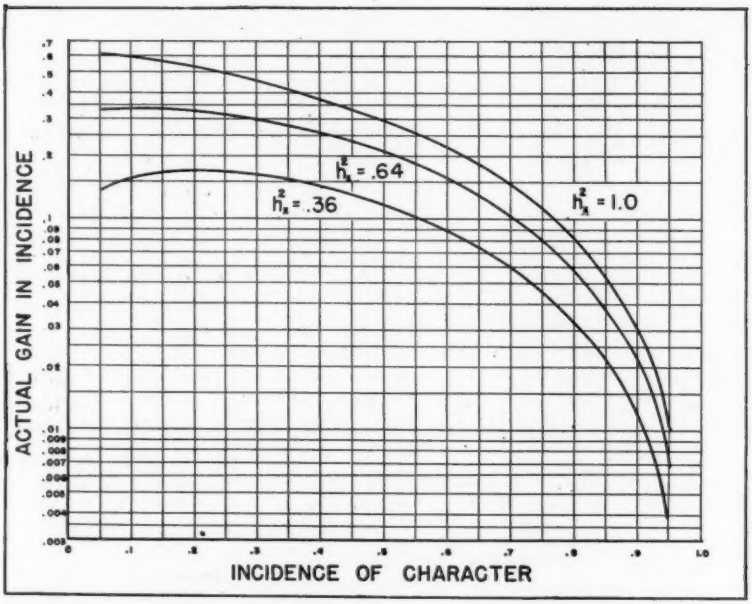

FIGURE 5. Actual gain in character incidence due to use as parents of all individuals exhibiting the character, expressed as a function of character incidence in the population.

dom sample of them) be used as the parents of the next generation, the mean genetic advantage of the selected animals and hence the genetic gain on the x scale is $h_x^2\bar{z}/p_p$. Subtraction of this gain from the distance between the threshold and the mean of the parental generation gives the corresponding threshold distance for the offspring. The area to the right of this threshold is then the incidence of the character in the offspring (p_0) and the difference $p_0 - p_p$ is the genetic gain on the p scale.

The gains thus computed under the conditions noted are illustrated in figure 5. It may be seen that mass selection *for* a character of high incidence (or *against* one of low incidence) leads to slight gains only. This is due to very low effective heritability, combined with the fact that selection intensity here

is also perforce low. For intermediate incidence the gains obtained are relatively high, but further shift in incidence does not produce much greater gains, because the decrease in effective heritability offsets and eventually overcomes the increase in selection intensity incident on this shift.

The percentage by which the gains computed on the basis of additive heritabilities, and additive plus a quarter epistatic heritabilities, under- or

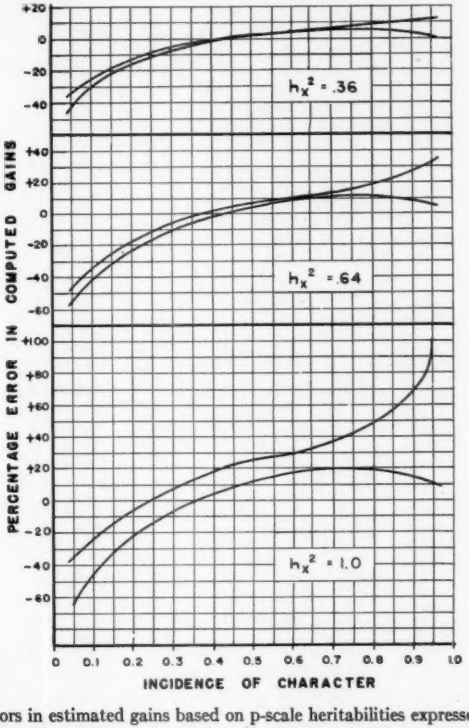

FIGURE 6. Errors in estimated gains based on p-scale heritabilities expressed as a percentage of actual gains, for the case where all individuals exhibiting the character are used as parents. The lower curve for each value of h_x^2 is based on additive heritability and the upper curve on additive plus one quarter of epistatic heritability.

over-estimate the actual gains are shown in figure 6. It will be noted that the actual rate of gain in the selection for a rare character may be more than twice that calculated on the basis of p scale heritabilities. On the other hand, in the case of characters that are already very frequent in the population the rate of gain is likely to be somewhat less than that calculated, but the discrepancy is relatively small except where the epistatic variance is very great. High epistatic variance, on the model discussed, occurs only where the heritability on the x scale is high or the incidence is very close to zero or unity.

If the selection intensity for the character is lower than the proportion of

individuals exhibiting it, it is necessary to use as parents of the next generation some individuals that do not exhibit the character. In this case the gain computed on the basis of p scale heritabilities is the weighted mean of the products of the heritability by the phenotypic advantages on the p scale of individuals exhibiting and not exhibiting the character. The latter values are

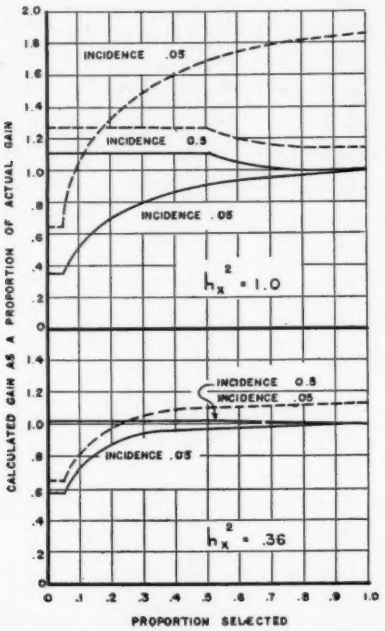

FIGURE 7. Errors in estimated gains based on p-scale heritabilities expressed as a proportion of actual gains, for cases where all individuals exhibiting the character and various proportions of individuals not exhibiting the character are used as parents. The solid line curve for each value of h_x^2 is based on additive heritability and the broken curve on additive plus one quarter of epistatic heritability. The two curves for $h_x^2 = .36$ and $p = .5$ are indistinguishable from each other on the scale portrayed.

of course $1-\bar{p}$ and $\bar{p}$ respectively, while the weighting is the proportion of parental individuals falling into the two classes. In computing the actual gain, the change in threshold value is computed in a similar fashion. Figure 7 shows the discrepancies between the actual gains and those expected on the basis of the p scale for certain cases where all individuals in the population exhibiting the character and various proportions not exhibiting the character are used as parents. These curves, as well as those in figure 6, suggest that calculations on the basis of additive heritability will always yield accurate estimates of the gain where the selection intensity is very low.

THE EFFICIENCY OF MASS SELECTION OF THRESHOLD CHARACTERS

If the proportion of individuals required as parents is exactly equal to the incidence of the character in the population so that all such individuals and no others are used as parents in a mass selection program, the ensuing improvement is exactly the same, in the case of the model being discussed, as

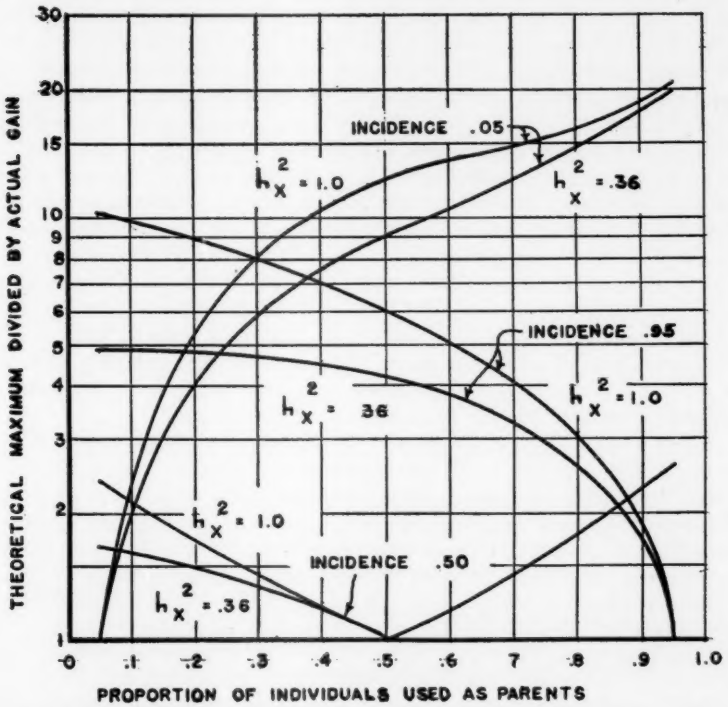

FIGURE 8. Gains that can be achieved on the basis of the all-or-none classification expressed as the reciprocal of the proportion of the gains that could theoretically be achieved on the basis of the underlying variate, for different proportions of individuals in the population that are used as parents.

though selection were based directly on the underlying variate. On the other hand, selection directly on the basis of the underlying variate would produce greater gains when a larger number of parents is required than the number of animals in the population which exhibit the character, or when a smaller number than that exhibiting the character is selected at random. The proportion of the maximum gains theoretically obtainable which are realized when selection on the p scale is practiced is shown in figure 8 for a series of different conditions.

It may be seen that under some circumstances, notably where strong selec-

tion can be applied in favor of a very common character (corresponding to the case of strong selection against a rare defect), or weak selection for a rare character, selection on the all-or-none basis is exceedingly inefficient. In such cases some indication of relative genetic values among individuals exhibiting or not exhibiting the character, as the case may be, would be valuable in improving the efficiency of mass selection. An example of an attempt to improve selection efficiency by such means is that of LUSH, LAMOREUX and HAZEL (1948)[3] to utilize age at death, rather than merely the fact of death during the first production year, as an indication of genetic value for viability. Where such attempt is unsuccessful, as in the case of the authors just cited, family selection may be many times as effective as mass selection and the utility of the latter may be negligible by contrast.

The errors resulting from the use of heritability on the p scale would, in general, be of less importance in family selection than in individual selection. The larger the number of individuals per family, the less important would be the discrepancy between computed and attained gains. One reason why this is true is that the magnitude of that portion of the genetic variance of family means attributable to genetic variance within families, a portion which includes most of the epistatic variance, is only (1/n) times as great as the within family genetic variance. Another reason is that environmental variance of different families does not cover as wide a range because the total genetic variance of family means is less than that of individuals.

In combined individual and family selection, therefore, extra weight should be assigned to the individual in those situations in which actual gains from mass selection are greater than those calculated on the basis of h_p^2 (see figure 6); conversely extra weight should be given to the family where expected gains from mass selection are less than calculated. Calculation of the precise allowances to be made are beyond the scope of this paper but would be useful to the extent to which the model being investigated is approximated by actual cases of economically important threshold characters.

SUMMARY

A convenient scale of measurement of the genotype of individuals with respect to all-or-none polygenic characters is that of the *probability of expression* of the character in relation to the total array of environments. On such a scale, however, the genetic variance is almost certain to be partly, and under some circumstances is largely, non-additive in nature. In addition the environmental variance, expressed on the same scale, is not independent of the genotypic level. An investigation is made of the effects of these disturbing features on estimates of gain from mass selection computed on the basis of heritability determinations.

On the basis of a simple mathematical model the non-genetic variance is shown to vary from a negligible proportion of the total genetic variance to

[3] An excellent discussion of the principles of selection for all-or-none traits will be found in the concluding sections of the paper by LUSH, LAMOREUX and HAZEL. It is oriented on what we have called the expected rather than the actual gains in our previous section.

more than three quarters of the total for the range of circumstances considered (figure 3). Only a small proportion of the non-additive variance would usually be included in heritability estimates based on full and half sib correlations (figure 4). Estimates of gain calculated on the basis of heritability determinations would constitute good approximations of the gain that should actually be achieved where the heritability of the variate that is postulated to underlie the appearance of the character is moderately low and the character incidence is not too close to zero or unity; under other conditions the gain may be grossly over- or under-estimated (figure 6). Gains calculated on the basis of the additive heritability, for all the situations studied, are accurate where the selection pressure is very low (figure 7). Under some circumstances gains could be achieved at a much greater rate could classification of the phenotype be made on a graduated rather than on an all-or-none basis (figure 8).

LITERATURE CITED

BLISS, C. I., 1935 The calculation of the dosage mortality curve. Ann. appl. Biol. **22:** 134–167.

FINNEY, D. J., 1947 Probit analysis. xiii+256 pp. Cambridge: University Press.

FISHER, R. A., 1918 The correlation between relatives on the supposition of Mendelian inheritance. Trans. roy. Soc. Edinb. **52:** 399–433.

FISHER, R. A., F. R. IMMER, and O. TEDIN, 1932 The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. Genetics **17:** 107–124.

HAZEL, L. N., 1943 The genetic basis for constructing selection indexes. Genetics **28:** 476–490.

JOHANNSEN, W., 1903 Erblichkeit in Populationen und in reinen Linien. 68 pp. Jena: Fischer.

LERNER, I. M., and D. CRUDEN, 1948 The heritability of accumulative monthly and of annual egg production. Poult. Sci. **27:** 67–78.

LUSH, J. L., 1945 Animal Breeding Plans. 3rd ed. viii+443 pp. Ames, Iowa: Collegiate Press.

1947 Family merit and individual merit as bases for selection. Amer. Nat. 81: 241–261, 362–379.

LUSH, J. L., W. F. LAMOREUX and L. N. HAZEL, 1948 The heritability of resistance to death in the fowl. Poult. Sci. **27:** 375–388.

ROBERTSON, A., and I. M. LERNER, 1949 The heritability of all-or-none traits: viability of poultry. Genetics **34:** 395–411.

SMITH, H. FAIRFIELD, 1936 A discriminant function for plant selection. Ann. Eugenics **7:** 240–250.

STERN, C., 1943 The Hardy-Weinberg law. Science **97:** 137–138.

WEINBERG, W., 1909 Über Vererbungsgesetze beim Menschen. Z. indukt. Abstamm. u. Vererblehre. **1:** 377–392, 440–460; **2:** 276–330.

1910 Weitere Beiträge zur Theorie der Vererbung. Arch Rass. -u. GesBiol. **7:** 35–49, 169–173.

WRIGHT, S., 1917 The average correlation within subgroups of a population. J. Wash. Acad. Sci. **7:** 532–535.

1918 On the nature of size factors. Genetics **3:** 367–374.

1920 The relative importance of heredity and environment in determining the piebald pattern of guinea pigs. Proc. nat. Acad. Sci. **6:** 320–332.

1921 Correlation and causation. J. agric. Res. **20:** 557–585.

1926 A frequency curve adapted to variation in percentage occurrence. J. Amer. statist. Ass. **21:** 162–178.

1934a The method of path coeffcients. Ann. math. Statist. **5:** 161–215.

1934b An analysis of variability in number of digits in an inbred strain of guinea pigs. Genetics **19:** 506–536.

1934c The results of crosses between inbred strains of guinea pigs, differing in number of digits. Genetics **19:** 537–551.

1935 The analysis of variance and the correlations between relatives with respect to deviations from an optimum. J. Genet. **30**: 243–256.

1943 An analysis of local variability of flower color in *Linanthus parryae*. Genetics **28**: 139–156.

YULE, G. U., 1906 On the theory of inheritance of quantitative compound characters on the basis of Mendel's laws—a preliminary note. Rep. 3rd Int. Conf. Genetics: 140–142.

APPENDIX

PROOF THAT THE ADDITIVE HERITABILITY ON THE P SCALE IS GIVEN BY THE EXPRESSION $\bar{z}^2 h_x^2/\bar{p}\bar{q}$.

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As indicated in the text, we may expect the additive genetic value on the p scale, (G_{pa}) of a genotype having the value G_x on the x scale to be a linear function of x. We may write, therefore:

$$G_{pa} = a + bG_x.$$

Since G_{pa} is to be a least squares estimate of the genotypic value on the p scale, G_{pt}, b is the linear regression coefficient of G_{pt} on G_x. Further the variance of G_{pa} will equal the variance of G_x multiplied by b^2, or:

$$\sigma_{G_{pa}}^2 = b^2\sigma_{G_x}^2.$$

The linear regression coefficient, as is well known, is the covariance divided by the variance of the independent variate, so the expression above may be written simply:

$$\sigma_{G_{pa}}^2 = \frac{\mathrm{cov}^2\,(G_xG_{pt})}{\sigma_{G_x}^4}\sigma_{G_x}^2 = \left(\frac{\mathrm{cov}\,(G_xG_{pt})}{\sigma_{G_x}}\right)^2. \tag{1}$$

The value G_{pt} corresponding to any value G_x may be written as follows:

$$G_{pt} = \frac{1}{\sqrt{2\pi}\,\sigma_E}\int_{t-G_x}^{\infty} e^{-x^2/2\sigma_E^2}dx \tag{2}$$

where t is the theshold value and G_x has the frequency distribution:

$$f(G_x) = \frac{1}{\sqrt{2\pi}\,\sigma_{G_x}} e^{-G_x^2/2\sigma_{G_x}^2}. \tag{3}$$

To simplify the symbols we may write:

$$G_x = x_1 \quad \text{and} \quad \sigma_{G_x} = \sigma_1.$$

The covariance may then be written as follows:

* The proof has been slightly altered and abbreviated from MR. ROBERTSON'S version. The authors, who had rested with only an empirical verification of the relationship here developed, are greatly indebted to MR. ROBERTSON for this demonstration.

$$\text{cov} = M(x_1 G_{pt}) = \frac{\int_{-\infty}^{+\infty} f(x_1) x_1 G_{pt} dx_1}{\int_{-\infty}^{+\infty} f(x_1) dx_1}.$$

The denominator equals unity. The numerator may be integrated by parts, letting $f(x_1)x_1dx_1 = dv$ and $G_{pt} = u$. Then:

$$v = \int \frac{x_1}{\sqrt{2\pi}\,\sigma_1} e^{-x_1^2/2\sigma_1^2} dx_1 = -\sigma_1^2 f(x_1)$$

and

$$du = dG_{pt}$$

and since the upper limit of expression (2) is fixed, this equals:

$$du = \frac{1}{\sqrt{2\pi}\,\sigma_E} e^{-(t-x_1)^2/2\sigma_E^2} dx_1.$$

Therefore:

$$\text{cov} = [uv]_{-\infty}^{+\infty} - \int_{-\infty}^{+\infty} v\,du$$

$$= -[G_{pt}\sigma_1^2 f(x_1)]_{-\infty}^{+\infty} - \int_{-\infty}^{+\infty} \frac{-\sigma_1^2 f(x_1)}{\sqrt{2\pi}\,\sigma_E} e^{-(t-x_1)^2/2\sigma_E^2} dx_1$$

and since the first term is zero at both limits this equals (substituting from (3))

$$\frac{\sigma_1}{2\pi\sigma_E} \int_{-\infty}^{+\infty} e^{-A/2} dx_1 \qquad (4)$$

where

$$A = \frac{x_1^2}{\sigma_1^2} + \frac{(t - x_1)^2}{\sigma_E^2} = \frac{x_1^2}{\sigma_1^2} + \frac{1}{\sigma_E^2}(t^2 - 2tx_1 + x_1^2)$$

$$= \frac{1}{\sigma_E^2}\left[\frac{\sigma_1^2 + \sigma_E^2}{\sigma_1^2} x_1^2 - 2tx_1 + t^2\right]$$

which may be written, since $\sigma_1^2 + \sigma_E^2 = 1$

$$= \frac{1}{\sigma_E^2}\left[\frac{x_1}{\sigma_1} - t\sigma_1\right]^2 + \frac{1}{\sigma_E^2}[t^2 - t^2\sigma_1^2]$$

$$= \frac{1}{\sigma_1^2\sigma_E^2}(x_1 - t\sigma_1^2)^2 + t^2.$$

Substituting in (4):

$$\text{cov} = \frac{\sigma_1}{2\pi\sigma_E} e^{-t^2/2} \int_{-\infty}^{+\infty} e^{-(x_1 - t^2\sigma_1^2)^2/2\sigma_1^2\sigma_E^2} dx_1$$

$$= \frac{\sigma_1}{2\pi\sigma_E} e^{-t^2/2} \sqrt{2\pi}\, \sigma_1\sigma_E = \sigma_1^2 \frac{1}{\sqrt{2\pi}} e^{-t^2/2} = \bar{z}\sigma_1^2.$$

Substituting in (1)

$$\sigma_{G_{pa}}^2 = \left(\frac{\bar{z}\sigma_1^2}{\sigma_1^2}\right)^2 = \bar{z}^2\sigma_1^2 = \bar{z}^2 h_x^2.$$

Dividing the additive genetic variance by the total genetic variance:

$$h_{pa}^2 = \frac{\bar{z}^2 h_x^2}{\bar{p}\bar{q}}.$$

THE EFFECT OF IRRADIATED MEDIUM, CYANIDE AND PEROXIDE ON THE MUTATION RATE IN NEUROSPORA

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STONE, WYSS, and HAAS (1947) and STONE, HAAS, CLARK, and WYSS (1948) have demonstrated that nutrient broth irradiated with ultra-violet light prior to inoculation of *Staphylococcus aureus* induces mutation in this organism. The rate of mutation of *S. aureus* to penicillin and streptomycin resistance and to inability to ferment mannitol can be increased by this method of indirect treatment of the bacterium with ultra-violet light.

The discovery of the effect of irradiated medium upon bacteria has opened up a new avenue of attack upon the problem of induction of mutation and possibly mutation itself. It would be of some interest therefore, to extend this method of treatment to an organism other than Staphylococcus, preferably one in which a sexual cycle is easily demonstrated so that the mutations which are derived can be shown to fulfill the more conservative definitions of a mutation. The organism should also be one in which the gametes are easily exposed to a medium containing chemical compounds with a relatively short lifetime, and which lends itself to the analysis of mutation rates. Neurospora was chosen as the test organism because of its easily controlled sex cycle, and the fact that the gametic nuclei are easily exposed to treatment with chemicals just as are the cells of a bacterium.

In addition to testing the effects of irradiated medium on Neurospora, experiments were also performed using hydrogen peroxide and potassium cyanide. WYSS, CLARK, HAAS, and STONE (1948) have reported results which indicate that hydrogen peroxide may play an important role in the effect of the irradiated medium on the mutation rate. If this is so, then one might expect cyanide to be effective, since it is a known inhibitor of the hydrogen peroxide decomposing enzyme, catalase. Theoretically, poisoning the catalase should permit the peroxide concentration in the cell to build up to a high enough level to have a mutagenic effect.

GENERAL METHODS AND MATERIALS

The wild type strains of *Neurospora crassa* used in all of these experiments were derived from single ascospore cultures obtained from a cross between Emerson wild types 5297a and 5256A. All irradiation was done with a Hanovia double-U, SC-2537 ultra-violet mercury vapor lamp operating at 120 milliamperes. The material was irradiated at a distance of 15 cm from the lamp.

Difco nutrient broth such as used in experiments described by STONE

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et al. (1947, 1948) was irradiated for periods of time ranging from 30 to 120 minutes. Conidia and in a few cases protoperithecia were added to this medium and allowed to stand immersed in it for three to 12 hours. When the mycelium was treated, it was immersed for three days in the irradiated medium. After immersion the material was centrifuged and washed twice with distilled water, by centrifugation, and then mated to the opposite sex on corn meal agar or WESTERGAARD'S medium (WESTERGAARD and MITCHELL 1947). Essentially the same procedure was used to treat the conidia with 0.05 percent potassium cyanide and 2.5 to 5.0 parts per million of hydrogen peroxide before mating.

Ascospores from the crosses were isolated and tested for biochemical mutations using the technique of BEADLE and TATUM (1945) and also by means of the method described by LEIN, MITCHELL and HOULAHAN (1948) for the selection of biochemical mutants of Neurospora. Relevant details concerning the application of these methods are given in the experimental section below.

EXPERIMENTAL PROCEDURE

Ascospores Picked at Random

The ascospores from the crosses involving treated conidia, protoperithecia or mycelium were isolated directly from the dissected perithecia, one spore being taken from each perithecium (HOROWITZ, HOULAHAN, HUNGATE, and WRIGHT 1946). Crosses were made using Neurospora material treated with medium irradiated for 30 or 120 minutes. The duration of treatment with the medium was 3 or 12 hours for the conidia and protoperithecia, and three days for the mycelium. Controls were carried out in which the conidia, protoperithecia and mycelium were treated with unirradiated medium for corresponding periods of time. All ascospores isolated from these crosses were transferred to a complete medium consisting of: Difco yeast extract, 2.5 gm; malt extract, 2.5 gm; casein hydrolysate, 200 mg; and a vitamin solution containing the known B vitamins, all dissolved in 1 liter of the standard Neurospora minimal medium (BEADLE and TATUM 1945). The cultures which grew on complete medium were tested for the occurrence of biochemical mutants, and also closely examined for morphological mutants.

Ascospores Selected after Germination

In this group of experiments in which the LEIN, MITCHELL, HOULAHAN technique was employed, only the conidia were treated, and the nutrient medium was in all cases irradiated for 120 minutes. The treatment with cyanide and hydrogen peroxide was carried out both in the presence and absence of nutrient medium. In the case of the cyanide the medium had been previously irradiated, but the peroxide was used in the presence of unirradiated medium. The concentration of conidia was carefully controlled during the course of treatment with the active agents, being in general about 10^7/ml. In all cases the conidia were treated for 12 hours, and after being washed used to fertilize protoperithecia which had previously matured on solid WESTER-

GAARD'S medium in Petri plates. The plates were then incubated at 25°C until the perithecia matured and had started to emit ascospores spontaneously at which time they were inverted over other plates containing solid minimal medium. The ascospores collected on the minimal plates were heat treated and incubated at 25°C for 15 hours. Ascospores were collected from perithecia at daily intervals from the time they started to emit spores until they ceased emitting, usually a period of about ten days. At the end of this period many of the spores had germinated and appeared as illustrated in figure 1. Over 95 percent of the germinated spores were of type D or had proliferated even

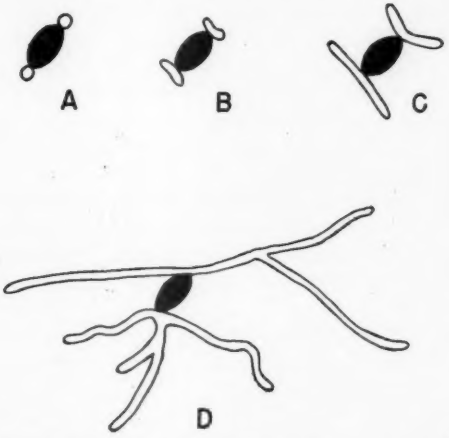

FIGURE 1. Appearance of germinated ascospores after 15 hours on minimal medium. Types A and B were picked as mutant; the others were not picked.

more than illustrated. Those spores which had the appearance of A or B in figure 1 were considered to be possible mutants and were picked from the plates with a platinum spatula. In order to prevent as much as possible picking replicate mutants from the same zygote, the following procedure was used. If two or more type A or B spores were observed lying close together, only one was picked. Furthermore each culture derived from the picked spores was marked with the number of the plate from which it was picked for a later check when the mutants were identified. (See LEIN, MITCHELL and HOULAHAN (1948) for a complete discussion of this technique and evidence of its utility in detecting biochemical mutants.) The total number of spores on each plate was counted, and the number which had germinated determined. Those which were picked as mutant were cultured on complete medium and subsequently tested for the presence of biochemical mutations. The complete medium used was somewhat modified from the one described for the previous experiments. It contained Difco yeast extract, 2.5 gm; Difco bacto-peptone, 5.0 gm; Difco beef extract, 3.0 gm; Neurospora extract, 0.1 liter; minimal medium,

0.9 liter. The Neurospora extract was substantially the same as that described by LEIN, MITCHELL and HOULAHAN (1948).

Two types of control crosses were made: one in which untreated conidia were used, and another in which the conidia were treated with unirradiated medium for 12 hours and otherwise handled exactly like those which were treated with irradiated medium. Conidia were also treated directly with ultra-violet light for 60 seconds at distances of 15 and 8.5 cm.

The procedure used for testing the cultures derived from ascospores picked as mutant was as follows. Each culture was inoculated into 25 ml of minimal medium and incubated for 72 hours. The mycelium, if one developed, was dried and weighed. Those cultures which did not grow on minimal or which grew slowly, that is, less than 50 per cent of wild type controls, were retained for further testing. All others were discarded after being examined for the occurrence of morphological mutants. The cultures which grew slowly or not at all on minimal were tested on complete medium and mixtures of the usual vitamins, amino acids and purines and pyrimidines. Any cultures which responded to the complete medium or the solutions of known growth factors by giving growth approximating that of wild type were considered as biochemical mutants, and tested for further identification. Those cultures which did not respond were considered as "slow growing mutants." In all of these cases growth was consistently less than 15 percent of wild type on minimal medium.

RESULTS

Effect of Irradiated Medium as Determined by Picking Ascospores at Random

Table 1 presents the results obtained by using the random picking technique. The data indicate an increase in the mutation rate due to treatment of the conidia with irradiated medium. Neglecting the morphological mutants, because of the high degree of subjectivity involved in their determination, and considering only the biochemical mutants, it will be noted that none of these

TABLE 1

The mutation rate after treatment with irradiated medium compared to the spontaneous rate. Ascopores picked at random.

TREATMENT OF MEDIUM	PART TREATED	CONTACT TIME	NO. TESTED ISOLATIONS	NO. MORPHOLOGICAL MUTANTS	NO. BIOCHEMICAL MUTANTS	PERCENT BIOCHEMICAL MUTANTS	L.E. (P=0.05)
None	Conidia and protoperithecia	3 to 12 hours	1,057	2	0	—	—
None	Mycelium	3 days	205	0	0	—	—
TOTALS			1,262	2	0	0	0.29–0
Irradiated 30 min.	Conidia and protoperithecia	3 hours	842	4	1	—	—
Irradiated 30 min.	Conidia	12 hours	222	0	1	—	—
Irradiated 30 min.	Mycelium	3 days	205	1	0	—	—
Irradiated 120 min.	Mycelium	3 days	203	1	2	—	—
TOTALS			1,479	6	4	0.27	0.69–0.073

TABLE 2

The spontaneous biochemical mutation rate.

NO. CULTURES TESTED	NO. BIOCHEMICAL MUTANTS	SOURCE
276	0	EMERSON (1944)
760	1*	HOROWITZ, HOULAHAN, HUNGATE and WRIGHT (1946)
697	0	MCELROY, CUSHING and MILLER (1947)
3,000	1	TATUM, BARRATT, FRIES and BONNER (in press)
1,262	0	This paper
5,995	2	TOTALS

* Doubtful biochemical mutant.

occurred in the controls, but that four were detected in the treated samples. The limits of expectation, however, given in the last column of the table for the total mutation frequency show that the rate of 0.27 percent for the biochemical mutants is not significantly different from the zero rate obtained in the controls. [The limits of expectation appearing in table 1 and insubsequent tables are calculated using the Table of Binomial and Poisson distributions as prepared by STEVENS (1942) with $P=0.05$.]

Various workers have presented data in the literature relating to the spontaneous biochemical mutation rate in Neurospora. These data have been collected and are given together in table 2. The totals of these together with the data from this paper give a spontaneous biochemical mutation frequency of 2/5,995 or 0.033 percent, and the limits of error in this case are 0.12–0.004. These added data on the spontaneous rate make the difference between the rate induced by the irradiated medium and the normal rate seem significant, but not decisively so. At best the data in table 1 should only be taken as an indication that the irradiated medium has a mutagenic effect.

Effects of Mutagenic Agents as Determined by Selecting Ascospores after Germination

The main body of the data relating to the effects of direct irradiation, irradiated medium, cyanide and hydrogen peroxide as determined by the use of the LEIN, MITCHELL, HOULAHAN technique appears in table 3. The percentage germination was consistently lower than the controls in all cases in which the conidia were treated by the agents mentioned above. None of the germinated ascospores from the control crosses that was inspected had the appearance of types A and B in figure 1, but a relatively high frequency of these types were observed after treatment with all the agents employed. Not all of the "mutant spores" transferred to complete medium continued growth beyond the stage developed on minimal. Therefore only those numbers of suspected mutants given in column 5 of the table were available for mutation tests. The total numbers of mutants determined, as given in column 6 includes

TABLE 3

Effect of direct irradiation, irradiated medium, cyanide and hydrogen peroxide. Ascospores selected after germination.

TYPE OF TREATMENT	(1) TOTAL ASCOSPORES	(2) TOTAL GERMINATED	(3) PERCENT GERMINATED	(4) NUMBER PICKED AS MUTANT	(4) NUMBER OF CULTURES OBTAINED	(6) TOTAL MUTANTS DETERMINED	(7) PERCENT MUTANTS OF THOSE PICKED	(8) PERCENT MUTANTS OF TOTAL GERMINATED
None	11,682	6,945	59.4	0	0	0	0	0
Unirradiated medium	11,530	7,066	61.2	0	0	0	0	0
Direct irradiation at 15 cm	7,733	3,554	46.0	148	75	30	40.0	0.84
Direct irradiation at 8.5 cm	4,521	615	13.6	22	15	4	26.7	0.65
Irradiated medium 10^7 conidia/ml	28,197	13,745	48.7	255	230	21	9.10	0.15
Irradiated medium 10^5 conidia/ml	13,575	5,313	39.1	142	124	13	10.5	0.25
Cyanide	14,735	6,009	40.8	188	155	18	11.6	0.30
Cyanide+irradiated medium	12,792	4,328	33.2	68	55	10	18.2	0.23
H_2O_2+medium 10^7 conidia/ml	14,377	6,373	44.3	157	130	14	10.8	0.22
H_2O_2+medium 5×10^5 conidia/ml	14,343	6,939	48.4	198	185	16	8.7	0.23
H_2O_2+water 5×10^5 conidia/ml	18,682	8,193	43.8	210	190	20	10.5	0.24

TABLE 4

The biochemical mutation date induced by the various agents, as detected by selecting ascospores after germination.

TREATMENT	MUTATION FREQUENCY	PERCENT MUTATIONS (NONMORPHOLOGICAL)	LIMITS OF EXPECTATIONS (P=0.05)
None	0/24,011	0.00	0.015–0
Direct irradiation	23/ 3,554	0.65	0.79 –0.59
Irradiated medium, 10^7 conidia/ml	16/13,754	0.12	0.19 –0.070
Irradiated medium, 10^5 conidia/ml	11/ 5,313	0.21	0.37 –0.10
Cyanide	16/ 6,009	0.27	0.41 –0.17
Cyanide+irradiated medium	9/ 4,328	0.21	0.39 –0.095
H_2O_2+medium, 10^7 conidia/ml	14/ 6,373	0.22	0.37– 0.12
H_2O_2+medium, 10^5 conidia/ml	16/ 6,939	0.23	0.36– 0.15
H_2O_2+water, 10^5 conidia/ml	20/ 8,193	0.24	0.36 –0.16

both morphological and biochemical mutants. The percentages in the last column may be taken as the mutation rates obtained by the various treatments since they are the percentages of mutants detected among the total number of germinated ascospores inspected.

The percentages of biochemical and slow growing mutants detected are given in table 4 together with some additional control data. During the course of some preliminary observations 10,000 germinated ascospores from a cross involving untreated conidia were inspected with no type A or B ascospores observed. Forty-nine ascospores of type C were noted, however, but none

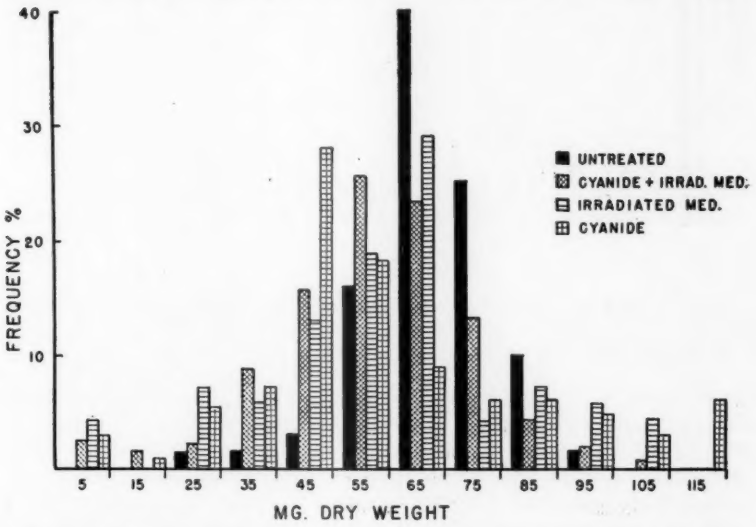

FIGURE 2. The weight distributions after 72 hours' growth on minimal medium of the cultures derived from ascospores selected after germination. The base line figures indicate the mid points of the various weight classes.

of these proved to be mutant after being tested. The mutation frequency for the control is given therefore as 0/24,011 in table 4. Comparing the percentages of biochemical mutations for the control and treated samples, and the limits of expectation calculated for each, it is evident that all of the various agents employed were effective in inducing mutation. The most effective agent was ultra-violet light used directly on the conidia. Irradiated medium used to treat conidia at a concentration of 10^7/ml was the least effective, while treatment with cyanide alone appeared to be the most effective among the chemical agents tested. However, since the limits of error calculated for the mutation percentages overlap for the samples treated with irradiated medium, cyanide and peroxide, very little can be concluded about the relative effectiveness of these agents.

The results obtained by determining the amount of growth attained on

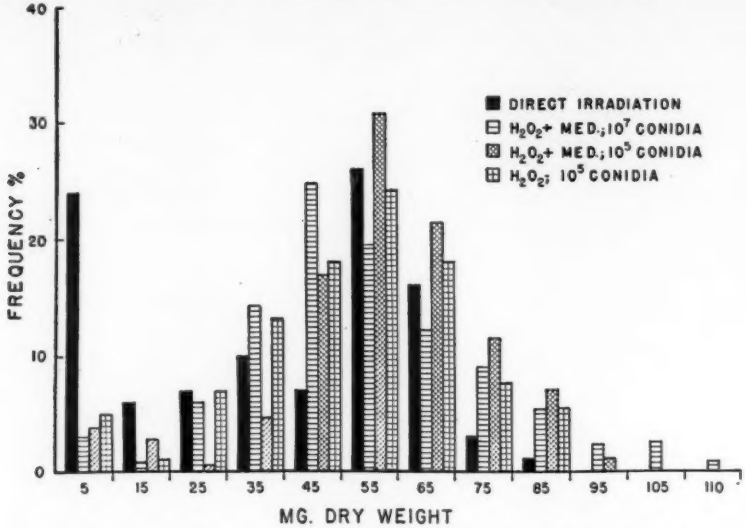

FIGURE 3. The weight distributions after 72 hours' growth on minimal medium of the cultures derived from ascospores selected after germination. The base line figures indicate the mid points of the various weight classes.

minimal medium by all the cultures derived from ascospores picked as mutant are illustrated in figures 2 and 3. Other data relating to these weight determinations are given in table 5. For purposes of comparison 200 cultures from germinated ascospores picked from the control plates were tested on minimal medium in the same way, and these results are included in figure 2 and table 5. The higher degree of variability of growth on minimal medium of the cultures selected from the treated crosses than found in the cultures selected at random from an untreated cross is evident.

TABLE 5

Variability of growth on minimal medium of cultures derived from ascospores selected after germination.

TYPE OF TREATMENT	MEAN DRY WEIGHT	STANDARD DEVIATION	COEFFICIENT OF VARIANCE
None	67.1	11.5	17.1
Direct irradiation	38.1	24.2	63.5
Irradiated medium	57.1	18.3	32.1
Cyanide	59.8	27.2	45.6
Cyanide+irradiated medium	58.6	22.7	38.7
H_2O_2+medium, 10^7 conidia/ml	54.0	20.2	37.4
H_2O_2+medium, 5×10^5 conidia/ml	56.5	18.0	31.9
H_2O_2+water, 5×10^5 conidia/ml	50.9	18.8	36.9

Identification of Mutants

The mutants detected in all of the experiments described above are tabulated in table 6. Only a few of the biochemical mutants have been identified positively as to their requirements for specific growth factors, and these, except in two cases are apparently of the usual type that have been described by previous investigators of Neurospora mutants. The two unusual cases occurred in stocks derived from conidia treated with irradiated medium. These grew only in the presence of a mixture of purines (xanthine, hypoxanthine,

TABLE 6

Identity of mutants detected.

TYPE OF TREATMENT	IDENTIFIED BIOCHEMICAL MUTANTS	UNIDENTIFIED BIOCHEMICAL MUTANTS	SLOW GROWING MUTANTS	MORPHOLOGICAL MUTANTS
Direct irradiation	thiamin methionine or threonine cytidine choline pyridoxin nicotinic acid tyrosine any amino acid	12	9	5
Irradiated medium	p-amino benzoic acid lysine purines and pyrimidines (2)	20	7	13
Cyanide	pyridoxin adenine	14	0	2
Cyanide and irradiated medium	0	10	0	0
Hydrogen peroxide	tryptophane lysine	39	8	1

adenine and guanine) and pyrimidines (cytidine, uracil, and thymine), but not on any of these singly, although there was slight growth on purines alone. The unidentified mutants are still in the process of being analyzed further.

Since there are a large number of unidentified mutants detected by the Lein, Mitchell, Houlahan technique there is a chance that some of these may be replicates, having some from the same zygote. No replicates were found among the identified biochemical mutants, but two cases of suspicious similarity among incompletely identified mutants picked from the same plate were observed and proper adjustment made for these in the tabulation of mutants in table 3. It is doubtful in view of the precautions taken whether the number of remaining replicates in the unidentified group, if there are such, significantly affects the mutation rate given as the true observed rate. LEIN,

MITCHELL and HOULAHAN (1948) report finding a very few cases of such replications among their identified mutants.

DISCUSSION

The first clear demonstration of artificial induction of mutations by means other than radiation directly applied to the cell was made by AUERBACH and ROBSON (1946) who used nitrogen and sulfur mustards to produce mutations in *Drosophila melanogaster*. Subsequent to this original work, HOROWITZ *et al.* (1946) and MCELROY *et al.* (1947) have shown that these agents are active in inducing biochemical mutations in *Neurospora crassa*. The results of these investigations have effectively created a new approach to the study of the causes of mutation which study had, in a sense, laid dormant for more than a decade previously due to the relatively narrow range of exploration afforded by the study of spontaneous mutation and the use of direct irradiation. But this new approach using chemicals as the active agents did not necessarily throw any light on the action of direct irradiation, and the two types of treatment could only be said to be acting in similar or dissimilar ways on the gene to bring about similar results. That a relationship does exist between the two types of treatment is demonstrated by the results of STONE *et al.* (1947, 1948) which show that mutations can be induced in bacteria by irradiating the medium to be used as a nutrient source by the bacterium. The further evidence by WYSS, STONE and CLARK (1947); WYSS, CLARK, HAAS and STONE (1948) that the hydrogen peroxide in the nutrient medium formed by the action of ultra-violet light is probably indirectly responsible for the mutations makes the relationship even clearer. According to WYSS *et al.* (1948) hydrogen peroxide is probably not the direct mutagenic agent, since treatment of the medium with hydrogen peroxide before inoculation and then inoculating after the hydrogen peroxide concentration is no longer measurable, gives the same results as treating the organism in the presence of both nutrient medium and peroxide. Presumably compounds such as organic peroxides formed in the presence of peroxide out of the nutrilite in the medium may be acting directly on the gene, or there may be a number of links in the chain leading up to the final effect on the gene which starts with hydrogen peroxide. The possible importance of organic peroxides formed indirectly or directly by irradiation of the medium or cell is indicated by the results of DICKEY, CLELAND and LOTZ (1949) who have demonstrated the effectiveness of certain organic peroxides in causing reverse mutations at an adenineless locus of Neurospora.

The results presented in this paper support the work of STONE *et al.* (1947, 1948) and WYSS *et al* (1947, 1948) on bacteria, by showing that irradiated medium and hydrogen peroxide can bring about inheritable changes in the sexually reproducing organism, Neurospora. The mutations produced, so far as they have been identified are of the type which have been obtained by direct irradiation, showing that the method of treatment is probably not specific for any one class of mutants. We may conclude therefore, that the phenomenon discovered by STONE, WYSS and HAAS (1947) is probably a

general one in so far as it makes clear the fact that it is not necessary to directly "hit" the gene with a particle of energy from a radiant source in order to cause the gene to mutate, but that an indirect effect on the medium, or on certain cell constituents other than the gene itself, may be in part responsible.

WYSS *et al.* (1948) gave evidence to show that the concentration of bacteria treated with irradiated medium was an important factor in producing mutations; high concentrations of cells, *i.e.* 10^7/ml give a rate similar to the control rate, whereas 10^6 cells/ml give a relatively high rate of mutation. The results presented in the present article for Neurospora conidia are in agreement with this original observation. It may well be, in fact, that a decrease in concentration below the lowest tried, 10^5 conidia/ml, may drastically increase the rate for Neurospora.

According to WYSS *et al.* (1948) washed bacterial cells treated with a solution of hydrogen peroxide do not mutate at a rate above normal. However, the mutation rate induced in the case of Neurospora is significantly higher than the normal rate whether a nutrient medium is present or not when hydrogen peroxide is used. No explanation can be given at the present time for this disagreement in the results obtained.

Using the hypothesis stated in the introduction to this paper, that it should be possible to induce mutations by poisoning the cellular catalase and cytochrome system, and thus permit the accumulation of hydrogen peroxide, WYSS *et al.* (1948) tried sublethal concentrations of sodium azide on *Staphylococcus aureus* and found a marked increase in the mutation rate. Similar results were obtained in the work reported here by treating the conidia with cyanide, a compound which is supposed to have an inhibitory effect on the iron-porphyrin type enzymes similar to that given by azide. An expected increase in mutation rate by treatment with both cyanide and irradiated medium did not materialize. This is rather difficult to harmonize with the explanation that it is the peroxide in the irradiated medium which is responsible for the mutagenic properties of the medium. However, the sample involved (see table 3) was a small one and the expected limits are broad in range.

The biochemical mutation rates obtained by treatment with irradiated medium, cyanide and hydrogen peroxide were all consistently and significantly lower than the rate obtained with direct irradiation (see table 4). The possible reasons for this may be two. First, the chemical agents are introduced into the cell from the outside, and their mutagenic potentialities may be considerably weakened thereby. This may be either because the chances of coming into close contact with the gene are decreased because of the initial distance, or because the agents themselves may be destroyed before they can be highly effective, as for example the decomposition of hydrogen peroxide by the cell's catalase. Direct irradiation of the cell with ultra-violet, on the other hand, would be expected to form the mutagens in close proximity to the gene. Second, the best substrate or substrates for the production of the most effective mutagenic agent by ultra-violet light may occur only within the cell, and therefore the cell itself must be irradiated to get the highest rates of mutation.

SUMMARY

Biochemical mutations have been produced in *Neurospora crassa* at a rate significantly higher than the control rate by treating the conidia with nutrient broth which had been previously irradiated with ultra-violet light, and also by treating the conidia directly with hydrogen peroxide and with potassium cyanide. The mutations were detected after being passed through the sex cycle. The rates induced by these various agents are not significantly different from one another, but are significantly lower than the rate induced by direct irradiation with ultra-violet. The types of biochemical mutants produced, as far as they have been identified, do not seem to be any different from those induced by direct irradiation. These results with Neurospora confirm in all important aspects the results obtained by STONE *et al.* (1947, 1948) and WYSS *et al.* (1948) using bacteria, and definitely establish the production of mutants by indirect irradiation, as well as by hydrogen peroxide which has been postulated to be one of the mutagenic agents formed as a result of irradiation.

LITERATURE CITED

AUERBACH, C., and J. M. ROBSON, 1946 The chemical production of mutations. Nature **157:** 302.

BEADLE, G. W., and E. L. TATUM, 1945 Neurospora II. Methods of producing and detecting mutations concerned with nutritional requirements. Amer. J. Bot. **32:** 678–686.

DICKEY, F. H., G. H. CLELAND and C. LOTZ, 1949 The rôle of organic peroxides in the induction of mutations. Proc. nat. Acad. Sci. **35:** 581–585.

EMERSON, S., 1944 The induction of mutations by antibodies. Proc. nat. Acad. Sci. **30:** 179–183.

HOROWITZ, N. H., M. B. HOULAHAN, M. G. HUNGATE and B. WRIGHT, 1946 Mustard gas mutations in Neurospora. Science **104:** 233–234.

LEIN, J., H. K. MITCHELL, and M. B. HOULAHAN, 1948 A method for selection of biochemical mutants of Neurospora. Proc. nat. Acad. Sci. **34:** 435–442.

MCELROY, W. D., J. E. CUSHING, and H. MILLER, 1947 The induction of biochemical mutations in *Neurospora crassa* by nitrogen mustard. J. cell. Comp. Physiol. **30:** 331–446.

STEVENS, W. L., 1942 Accuracy of mutation rates. J. Genet. **44:** 301–307.

STONE , W. S., O. WYSS, and F. HAAS, 1947 The production of mutations in *Staphylococcus aureus* by irradiation of the substrate. Proc. nat. Acad. Sci. **33:** 59–66.

STONE, W. S., F. HAAS, J. B. CLARK, and O. WYSS, 1948 The role of mutation and of selection in the frequency of mutants among microorganisms grown on irradiated substrate. Proc. nat. Acad. Sci. **34:** 142–149.

TATUM, E. L., R. W. BARRATT, N. FRIES, and D. M. BONNER, 1949 Amer. J. Bot. (in press).

WESTERGAARD, M., and H. K. MITCHELL, 1947 Neurospora V. A synthetic medium favoring sexual reproduction. Amer. J. Bot. **34:** 573–577.

WYSS, O., W. S. STONE, and J. B. CLARK, 1947 The production of mutations in *Staphylococcus aureus* by chemical treatment of the substrate. J. Bact. **54:** 767–772.

WYSS, O., J. B. CLARK, F. HAAS, and W. S. STONE, 1948 The role of peroxide in the biological effects of irradiated broth. J. Bact. **56:** 51–57.

THE TRANSFER OF MUTANT GENES FROM SMALL INVERSIONS

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THE complete analysis of the crossover relationships of the lozenge series of alleles in *Drosophila melanogaster* has been hindered by the location of the two alleles known to give unusual crossover products within the *dl-49* inversion of the X chromosome (OLIVER 1940). In order to test for crossing over between those alleles (lz^0 and lz^s) within the inversion and other alleles in an uninverted chromosome, it has been necessary to transfer the alleles in the *dl-49* inversion to a normal sequence. Double crossing over within the inverted segment of *dl-49*/+ heterozygotes, the most obvious means of effecting such a transfer, has not been observed in extensive tests by K. C. GREEN (unpubl.) who used autosomal inversions to increase crossing over in the X chromosome, or in triploid oocytes; a special method has been devised to accomplish the task.

It is evident that the net product of a double crossover within an inversion might be obtained if it were feasible to combine two single crossovers within the inversion occurring at different times. An effective double obtained in this way would be free of the limitation of interference which prevents the occurrence of true doubles. Single crossing over in inversion heterozygotes gives rise to chromatid bridges during the first anaphase of meiosis which cannot be recovered in the functional egg nucleus (STURTEVANT and BEADLE 1936). However, by combining *dl-49* with a second larger inversion, the single crossovers within the common inverted region give rise to duplication-deficiency chromosomes rather than bridges. These duplication-deficiency chromosomes, ordinarily inviable with a normal chromosome, may be saved if the complementary duplication-deficiency products of a single crossover get into the same egg nucleus, for the duplication on one chromosome will balance the deficiency on the other and *vice versa*. Ordinarily, chromatids participating in an exchange are directed to opposite poles at meiosis, but it has been shown by BRIDGES and ANDERSON (1925) that the exceptional diploid daughters (those receiving both X chromosomes from the mother and a Y chromosome from the father) of triploid females occasionally carry the complementary products of a single crossover. A line carrying the unbalanced chromosomes may be carried indefinitely, for such females will produce, by non disjunction, daughters also carrying the duplication-deficiency chromosomes, plus a Y chromosome from the father. An additional crossover within the common inverted segment in such a balanced line will reconstitute the initial sequences and will effectively transfer a section of chromosome from one se-

quence to the other. The section transferred to the larger inversion may be removed to a normal sequence by ordinary double crossing over.

METHOD

A triploid stock with free X chromosomes was mated in alternate generations to *In(1)dl-49, v lz*^{g} males[1] and to *In(1)sc*^{8}, *sc*^{8} *cv v f* males so that the resulting *v* triploids had two X chromosomes of one kind and one of the other. If the uninverted sequence of genes on the X chromosome is represented by the letters *A* to *M* serially, with the position of the lozenge locus at *G*, the inverted sequences may be represented as:

$$In(1)dl\text{-}49 = A\ B\ C\ D.I\ H\ lz^{g}\ F\ E.J\ K\ L\ M\text{-}$$

and

$$In(1)sc^{8} = A.L\ K\ J\ I\ H + F\ E\ D\ C\ B.M\text{-}$$

A single crossover in the common inverted region, *I* to *E*, of the two different X chromosomes, as for example, between *I* and *H*, gives rise to the duplication-deficiency chromosomes:

$$A\ B\ C\ D.I\ H + F\ E\ D\ C\ B.M\text{-}$$

and

$$A.L\ K\ J\ I\ H\ lz^{g}\ F\ E.J\ K\ L\ M\text{-}$$

These chromosomes would be inviable separately in a male or in combination with a normal chromosome in the female, but together, form a balanced heterozygote.

By mating the triploids to *B* males and selecting non-*B* non-*cv* and non-*lz*^{g} diploid daughters, individuals having the two differently inverted X chromosomes (or the complementary crossover products of the two) and a Y chromosome from the father are obtained. Since such F_1 females will generally carry non-crossover chromosomes, they must be tested by mating them to *B* and examining their progeny for regular offspring. The females carrying non-crossover chromosomes will produce more regular than non disjunctional offspring, but those which get the complementary crossovers from the triploid mother will yield only matroclinous daughters and patroclinous sons except when, rarely, a second crossover restores the original balanced complement of genes to each chromosome. These two alternatives may be differentiated by simple inspection of the progeny without counting. Once a female carrying the duplication-deficiency chromosomes has been obtained, a stock may be kept by selection of the matroclinous daughters for the next generation.

When a second crossover occurs within the common inverted segment of the duplication-deficiency chromosomes, the inverted sequences are restored. But

[1] The mutant genes used in these crosses are all on the X chromosome and have the following positions: *cv*=crossveinless, 13.7; *v*=vermilion, 33.0; *f*=forked, 56.7; *B*=Bar, 57.0; *lz*^{s} and *lz*^{g} =spectacled and glossy alleles of lozenge, respectively, 27.7. *In(1)sc*^{8}, an inversion of almost the entire X chromosome, carries the inseparable *scute*^{8} phenotype.

when this crossover is on the side of the mutant gene being transferred, other than the one where the original crossover occurred, the mutant gene is transferred from one sequence to the other. Thus, if, as had been assumed earlier, the first crossover was located to the left of the lozenge locus and the second to the right, the new chromosomes would be:

$$A.L\ K\ J\ I\ H\ lz^{g}\ F\ E\ D\ C\ B.M\text{-}$$

and

$$A\ B\ C\ D.I\ H + F\ E.J\ K\ L\ M\text{-}$$

The first chromosome represents $In(1)sc^{8}$ carrying the lozenge allele lz^{g}. The transfer of the mutant from the long inversion $In(1)sc^{8}$ to a normal sequence by ordinary double crossing over presents no difficulty.

RESULTS

Out of 234 exceptional diploid females mated to *B* males, two produced only matroclinous daughters and patroclinous sons. A more extensive test of their daughters mated again to *B* gave:

			Heterozygous	
	non-*B* ♀	*B* ♂	*B* ♀	other
1.	169	200	19	1 sc^{8} *cv v* lz^{g} ♂
2.	291	299	38	3 sc^{8} *cv v* lz^{g} *f* ♂♂; 1 sc^{8} *cv v f* ♂

The heterozygous *B* females, when tested, proved to carry a reconstituted inverted chromosome and the paternal X-chromosome carrying *B*. The males carrying sc^{8} and lz^{g} represent those cases where the second crossover was on the other side of lz^{g} from the first, thereby shifting the mutant gene out of *In(1)dl-49* into $In(1)sc^{8}$. The sc^{8} *cv v f* male represents the reconstitution of the original sc^{8} chromosome by a crossover of the same side of lz^{g} as the one which gave the original duplication-deficiency chromosomes. The number of male progeny carrying reconstituted X chromosomes is smaller than the number of females presumably because of the reduced viability of males with the four or five mutant genes used as markers.

The spectacled allele of lozenge (lz^{s}) has been removed from the *dl-49* inversion in the same way. Out of 550 tests of exceptional daughters of triploids carrying $In(1)sc^{8}$, sc^{8} *cv v f* and *In(1)dl-49*, lz^{s}, two cultures produced mostly non disjunctional offspring and in a subsequent generation the second crossover yielded chromosomes with lz^{s} in $In(1)sc^{8}$, from which it was transferred to a normal sequence.

These mutant alleles, disassociated from inverted sequences, have been used in tests with other lozenge alleles to determine the cause of the unusual crossover products produced by certain combinations of alleles (GREEN and GREEN 1949).

SUMMARY

By a series of steps, it has been possible to remove mutant genes (lozenge alleles) in *Drosophila melanogaster* from an inversion, *dl-49*, which did not per-

mit their removal by ordinary double crossing over. The steps involve (a) the formation of duplication-deficiency chromosomes by a single crossover within the common inverted segment of two different inversions; (b) the recovery of both complementary duplication-deficiency chromosomes by non disjunction in a triploid mother; (c) the reconstitution of the original sequences by a second crossover, in subsequent generations, which in some cases transfers the mutant from one inversion into the second larger inversion; and, (d) the transfer of the mutant from the large inversion into a normal sequence by double crossing over.

LITERATURE CITED

BRIDGES, C. B., and E. G. ANDERSON, 1925 Crossing over in the X chromosomes of triploid females of *Drosophila melanogaster*. Genetics **10**: 418–441.

GREEN, M. M. and K. C. GREEN, 1949 Crossing over between alleles at the lozenge locus in *Drosophila melanogaster*. Proc. nat. Acad. Sci. **35**: 586–591.

OLIVER, C. P., 1940 A reversion to wild type associated with crossing over in *Drosophila melanogaster*. Proc. nat. Acad. Sci. **26**: 452–454.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relation of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.

GENETIC CHANGES INDUCED BY EXPOSURE TO NITROGEN MUSTARD AND THEIR INHERITANCE IN PARAMECIUM AURELIA, VARIETY 4*,[1]

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IN RECENT years chemical induction of mutation has assumed considerable importance in experimental genetics, as evidenced particularly by the work of AUERBACH and ROBSON (1944, 1946, 1947a, 1947b), AUERBACH (1949a, b) and others. These workers, investigating mustard gases and related compounds found them to be potent mutagens in various organisms. One of the compounds shown by AUERBACH and ROBSON (1947b) to be mutagenic was methyl bis ($\beta\beta'$-dichloroethyl) amine, frequently referred to as nitrogen mustard. Nuclear changes induced by this compound have been investigated extensively in plants and reviewed by DARLINGTON and KOLLER (1947). The subject of chemical induction of mutation has recently been reviewed by AUERBACH (1949b). The results of investigations in this subject indicate that nitrogen mustard is effective in producing a multiplicity of changes, both in the genes and chromosomes, irrespective of the organism used.

Such extensive effects, as have been demonstrated on genes and chromosomes, suggest the possibility of comparable effects on cytoplasmic genetic factors. AUERBACH and ROBSON (1947a) found that untreated chromosomes, introduced into treated cytoplasm were unaffected, indicating that the chromosomes do not react to mustard through the cytoplasm. Inasmuch as the tests required three generations to complete, they do not eliminate the possibility that most of the immediate action of mustard may be through the cytoplasm. It does indicate, however, that the delayed action is not brought about through the action on the cytoplasm.

Paramecium aurelia, one of the few animals known to be favorable for study of cytoplasmic inheritance, offers opportunities for investigating the effects of chemicals on both cytoplasmic and nuclear genetic factors. The inactivating effect of nitrogen mustard on the cytoplasmic factor, kappa, in *Paramecium aurelia* has been investigated and reported elsewhere (GECKLER 1949). The

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present work reports the effects of nitrogen mustard on viability in variety 4 of this species, and makes a start towards discovering the physical bases of these effects, particularly as to whether they are nuclear or cytoplasmic or both.

It seems desirable to attempt to explain as many of the data obtained from the study of nitrogen mustard effects in Paramecium as possible on the basis of nuclear phenomena. Elucidation of the physical basis responsible for additional data, seemingly incompatible with a nuclear hypothesis, can then be attempted.

MATERIALS

A. *Stocks*

Two stocks of *Paramecium aurelia*, variety 4, have been employed. One is the killer stock 51 and the other is stock 186, which is approximately isogenic with stock 51 but contains the gene *k* instead of the gene *K* (SONNEBORN 1947).

B. *The mutagen*

Nitrogen mustard, methyl bis ($\beta\beta'$-dichloroethyl) amine was supplied, in the form of the hydrochloride salt, through the kindness of DR. RICHARD A. ORMSBEE of the SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH. Concentrations of nitrogen mustard used in this study are given in terms of the hydrochloride salt.

METHODS

A. *Exposure of animals to the action of nitrogen mustard*

Control animals were exposed to phosphate buffer (pH 6) alone while the experimental animals were exposed to buffer containing varying amounts of nitrogen mustard. To obtain animals for exposure an untreated clone was allowed to undergo autogamy and single autogamous individuals isolated. After these isolations had yielded small clones, consisting of four to sixty-four offspring, and within a half-hour after the last fission, one or two members of each clone were taken as controls and the remaining animals were exposed to the action of the mutagen as described below.

A quantity of nitrogen mustard hydrochloride (usually approximately 10 to 20 milligrams) was weighed and dissolved in a small quantity of the phosphate buffer. It was immediately made up to 50 ml in a volumetric flask and the animals exposed to its action within two minutes. No attempt was made to weigh an exact predetermined quantity. This accounts for the variations in concentration of nitrogen mustard used in the exposures throughout this report.

After thorough mixing of the solution of nitrogen mustard one drop was introduced into a drop of buffer containing the animals to be exposed. The two drops were mixed quickly by agitating the slide in which they were contained. After varying times animals in this solution were transferred with a micropipet in a minimum of fluid and discharged into a depression of fresh culture fluid. The fluid in the depression was mixed thoroughly; the animals were transferred

to a second and third depression and the procedure repeated. The animals were then isolated individually in fresh culture fluid in depression slides. Controls were subjected to this washing procedure after exposure to buffer alone.

If an experiment included more than one exposure time, all the animals to be exposed were placed in one exposing solution and different animals removed after varying times.

The temperature of exposures varied from 22° to 28°C. The numbers of exposures carried out at different temperatures were insufficient to demonstrate any correlation of degree of effect with temperature; temperatures are therefore not reported for the individual experiments.

B. *Induction of autogamy*

1. ***Tube method.*** Animals were isolated individually in depression slides in fresh culture fluid and placed at 27°C. After 4–8 fissions the cultures were transferred to test tubes containing approximately 15 ml of fresh culture fluid and were replaced at 27°C. Approximately three days later, the tubes cleared of bacteria and the animals formed a dense ring near the top of the column of culture fluid. This ring of animals was removed, placed in depression slides and allowed to starve. As the animals starve, they undergo autogamy and can be isolated. The stage of autogamy immediately following fertilization is known as the climax stage. The animals were isolated at this stage in order to avoid selection of the more vigorous animals. Some that are destined to be nonviable fail to reach later stages of nuclear reorganization, but none fails to reach the climax stage. Samples of the culture were stained periodically to ascertain when the climax stage was reached. At that stage the macronucleus is fully disintegrated into numerous fragments, but the new macronuclear anlagen have not yet formed. The animals are characteristically small, nearly round and pale but with a posterior black spot. The appearance of the living animals thus served as a convenient guide, but this was always checked by staining with acetocarmine. As most of the animals go through autogamy at about the same time under these conditions, the stained sample is a reliable index of the condition of the remaining animals. Climax animals were isolated almost exclusively throughout this investigation. However, if all the animals were in autogamy, as determined from a stained sample, and none appeared to be abnormal, isolations were made without reference to the stage of autogamy. Comparison of values of reduced viability, from a single clone, between samples of climax animals and animals isolated without reference to the stage of autogamy showed that the stage of autogamy made no difference in the fraction of clones of reduced viability, provided there were no visibly abnormal animals present in the autogamous clone at the time the samples were taken.

2. ***Daily isolation method.*** Single animals were isolated in depression slides and one cell from the progeny of each isolate was isolated daily into fresh culture fluid in another slide. The animals left over after each isolation had been made were allowed to starve and climax animals were isolated from these left-over cultures by the same methods as described for the tube method.

C. *Criteria for reduced viability*

Clones started as autogamous animals not exposed to nitrogen mustard, but isolated and allowed to grow for four days at 27°C in depression slides containing 1.5 ml of culture fluid will undergo not less than ten fissions and begin to starve. Approximately one percent of the clones fail to undergo this number of fissions; the number of fissions which they will undergo varies. Sometimes the isolated animals die without dividing, other times they may yield clones with any number of fissions up to eight or nine.

With rare exceptions, clones failing to undergo the usual number of fissions during the four day period, whether derived from treated or control animals, eventually die and are designated as clones of reduced viability. The four day period of growth before observation was selected because shorter periods fail to allow the classification of clones with sufficient accuracy while longer periods are no more accurate and are considerably inconvenient.

At the end of the four day growth period observations were recorded in the following manner. Clones that went through at least ten fissions were recorded as normal; the number of fissions was recorded if it was less than ten. A record was also made as to the occurrence of abnormal animals in any clone. Irrespective of abnormalities, clones were considered to be of reduced viability only if they went through less than ten fissions. This introduces some error because a clone that goes through ten fissions may be destined to die if it contains abnormal animals. As this happens only rarely, omission of such clones from the category of reduced viability fails to alter materially either the calculations or the conclusions.

The size of samples isolated from autogamous clones varied but is stated for each experiment.

Variations of these and other methods were used in some experiments and will be described in the appropriate sections.

EVIDENCE FOR THE MUTAGENIC ACTIVITY OF NITROGEN MUSTARD ON MICRONUCLEI

A. *Appearance of reduced viability at autogamy*

Table 1 shows the results of exposing 69 animals to the action of nitrogen mustard, inducing autogamy (by either method) in their vegative progeny, isolating samples of 24 autogamous animals and observing the resulting clones for reduced viability. It is apparent that the treated animals gave rise to many progeny of reduced viability after autogamy while the control animals gave rise to very few. Exposure to more concentrated solutions of nitrogen mustard results in still higher proportions of affected exautogamous clones.

Twenty-three of the 69 or 33 percent of the exposed animals gave reduced viability values exceeding the values for the controls in these three experiments. It is evident that the changes in viability at autogamy after exposure to nitrogen mustard are significantly different from the controls.

KIMBALL (1949) has shown that the number of mutations produced per micronucleus may be calculated by the expression $F = 1 - e^{-m/2}$ where m is the

TABLE 1. Experiments 1–3

Numbers of clones of reduced viability after autogamy. Samples of 24 autogamous animals were isolated from the progeny of animals exposed to nitrogen mustard. Entries in the table indicate numbers of treated animals.

EXPT. NO.	EXPOSURE		ANIMALS IN 24 ISOLATIONS AFFECTED														
	mg/ml	MIN.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1a	.211	10	2	2			1		1			2	1	1			1
1b	.211	11	1		2			1			1		1				2
1c	.211	11½	4			1	1										
1d	.211	12	5		1	1			1	1							
2	.215	5	16	4	1	1				1			2				
3	.158	3	7	1		1					1						
Controls		0	9	2	1												

mean number of mutations per micronucleus and F is the mean fraction of clones of reduced viability isolated at autogamy from a number of clones derived from rayed animals. F is calculated by converting the fraction of reduced viability in each sample to an angle using the table in SNEDECOR (1946), finding the arithmetic mean of the angles and converting the resulting mean angle back to a fraction. The equation involves the assumptions that the effects are completely expressed in homozygous condition, that all the animals derived from the original treated animal are genetically alike before autogamy and that all animals are equally liable to induction of genetic changes. If one assumes that the results shown in table 1 are due to genic effects, one may calculate the mean number of mutations which must have been produced in the micronuclei of the animals exposed to nitrogen mustard. The results of this calculation are shown in table 2.

TABLE 2. Experiments 1–3

Mean number of gene mutations per micronucleus calculated by Kimball's formula from the data given in table 1.

EXPT. NO.	NO. TREATED ANIMALS	F	MEAN NUMBER OF MUTATIONS PER MICRONUCLEUS
1a	11	0.193	0.42
1b	8	0.241	0.55
1c	6	0.017	0.03
1d	9	0.037	0.08
2	25	0.019	0.04
3	10	0.011	0.02

The values given in table 2 are acceptable only insofar as the assumptions concerning the equation are true. If some of the effects are non-genic, the calculations reveal nothing of the frequencies of each kind. In addition, as will be brought out in later sections, there is considerable doubt that animals of a clone are of identical genotype. In general, the data presented here are explicable on

a nuclear basis and are more or less what would be expected if the action of nitrogen mustard were on nuclear material.

B. *Failure to produce effects on viability during vegetative reproduction.*

In the experiments reported here 5.4 percent of the animals exposed to nitrogen mustard and six percent of the animals exposed to buffer alone failed to survive during subsequent vegetative reproduction. This failure to produce significant detectible effects during vegetative reproduction is in agreement with the known failure of the micronuclei to have phenotypic effects (SONNEBORN 1947) and is thus in keeping with expectations based on the hypothesis of micronuclear changes.

C. *Heterogeneous clones; segregation of the physical basis of reduced viability during vegetative reproduction*

During the course of detecting and analyzing effects of exposure to nitrogen mustard it was found that the proportion of affected animals at autogamy did not necessarily remain constant in subcultures of a clone derived from a single treated animal. The following experiments were performed to investigate this variation.

Experiment 4. Two two-fission exautogamous clones (A and B) were divided in half. Four animals, A1, A2, B1 and B2, were exposed to nitrogen mustard (0.242 mg/ml for 8 minutes) and four animals, AC1, AC2, BC1 and BC2, were exposed to buffer alone. After exposure the animals were separated and placed in different containers after each fission until, at the end of the third fission, each animal had given rise to eight sister animals (1–8) from which

TABLE 3. Experiment 4

Fraction of clones of reduced viability after autogamy in cultures derived from the third fission products after exposure to buffer or nitrogen mustard. Animals A1, A2 and B2 were exposed to nitrogen mustard. Animals AC1, AC2, BC1 and BC2 were exposed to buffer alone. Based on samples of 50 autogamous animals.

ANIMAL NUMBER	CULTURE NUMBER								χ^2	d.f.**	P***
	1	2	3	4	5	6	7	8			
A1	.36	.56	.02	.00	.38	.52	.76	.82	131.5	7	$<.01$
A2	.44	.34	.20	.06 .18*	.10	.04	.52	.50	74.4	8	$<.01$
B2	.86	—	.86	.94	.38	.30	.48	.38	94.1	6	$<.01$
AC1	.00	.02	.02	.00	.04	.04	.02	.00	3.0	7	$.80<P<.90$
AC2	.00	.00 .04*	.00	.00	.04	.08	.04	.02	18.0	8	$.01<P<.05$
BC1	.02	.08	.04	—	.02	.04	.04	—	6.7	5	$.20<P<.30$
BC2	.00	.00	.00	.00	.02	.00	.02	.00	6.0	7	$.50<P<.70$

* Expanded through 4 fissions.

** Degrees of freedom.

*** Probability that the sister cultures from a single treated animal are alike in their degree of reduced viability at autogamy.

eight separate cultures were grown. These cultures were grown, first in depression slides and later in test tubes, at maximum fission rate at 27°C and induced to go into autogamy by the tube method to preserve all the progeny from each culture. Samples of 50 climax autogamous animals were isolated individually from each culture and their viability recorded. Table 3 shows the fraction of autogamous clones of reduced viability found in each sample. Animal B1 died without undergoing fission. The other animals gave rise to viable vegetative clones until autogamy.

The results of experiment 4 indicate that large differences between subcultures derived from single treated animals may exist. Chi-square tests for homogeneity (SNEDECOR 1946) indicate that the data are heterogeneous, i.e. the probability is very small that all sister cultures from each treated animal are alike in their degrees of reduced viability. An interpretation of these differences may be made on the basis of delayed effects of nitrogen mustard. For example, consider the data obtained from the progeny of animal A1. The largest difference in reduced viability between descendants of the products of the third fission is shown by cultures 1 and 2. The difference of 0.20 in reduced viability is close to the limit of significance ($\chi^2=4.02$, $P=0.04$). From this it may be concluded that the differences between the values for cultures 1 and 2, cultures 3 and 4, cultures 5 and 6 and cultures 7 and 8 are explicable on the basis of sampling error. However, the mean of cultures 1 and 2 (0.46) differs significantly from the mean of cultures 3 and 4 (0.01). The mean of cultures 5 and 6 (0.47) differs significantly from the mean of cultures 7 and 8 (0.79). These means correspond to hypothetical values of reduced viability for the second fission products had they not been subcultured after the third fission. The means may be combined in a similar manner to obtain the hypothetical values for reduced viability after the second fission which in turn may be combined to give the value of reduced viability which would have been obtained had no subculturing been performed. These values have been plotted in figure 1 which also includes plots of the data obtained from the other exposed animals in experiment 4. By assuming the following, the data obtained from the progeny of animal A1 may be explained on the basis of delayed mutation. After exposure no change in the nuclear material occurred until after the first fission at which time mutations occurred in the animal destined to give rise to cultures 5–8. None occurred in the animal giving rise to cultures 1–4. After the second fission, additional mutations took place in the animal which gave rise to cultures 7 and 8 but no more occurred in the one which gave rise to cultures 5 and 6. In the animal giving rise to cultures 1 and 2 mutations took place after the second fission but none occurred in the one which gave rise to cultures 3 and 4. The cultures derived from third fission products fail to show significant differenences (0.95 probability) indicating that the process of mutation slowed considerably after this time.

Similar reasoning may be applied to the data obtained from the other animals exposed to nitrogen mustard.

The controls in experiment 4 show slight spontaneous changes, particularly the progeny from animal number AC2.

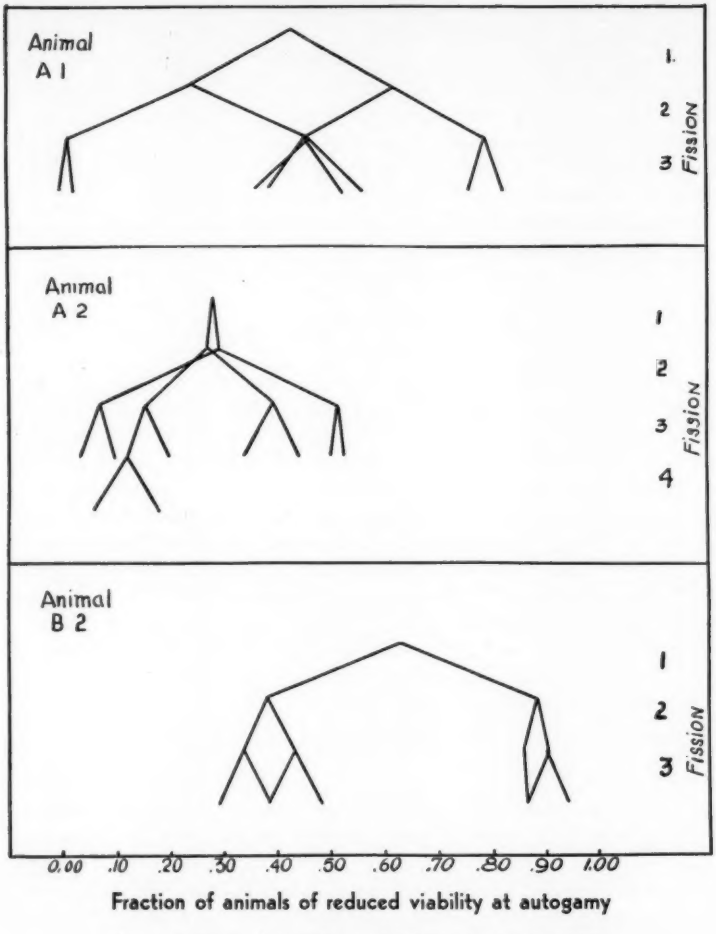

FIGURE 1. Segregation of the physical basis of reduced viability through vegetative reproduction

It must be emphasized that the foregoing explanation is formal and is based on the known property of nitrogen mustard of causing delayed mutations (AUERBACH and ROBSON 1947a).

Experiment 5. This experiment was performed to extend the investigation of possible differences between sub-clones derived from the same treated animal. Instead of isolating each fission product at each fission as in the previous experiment, the following procedure was used. After the first fission following exposure one fission product was isolated and allowed to multiply, all progeny

being saved. The other first fission product was allowed to undergo fission and the procedure repeated through the fifth fission. The fifth fission products were separated and allowed to give rise to separate clones. This procedure gives a total of six sub-clones from a single treated animal, one corresponding to each fission except the fifth. Two cultures were derived at the fifth fission. Autogamy was then induced in each sub-clone by the tube method. Eleven animals from a four fission exautogamous clone were exposed to 0.515 mg/ml nitrogen mustard for five minutes. Three animals from the same clone served as controls. Autogamy was obtained among the progeny of each of these 14 animals. The tube method was employed so as to minimize selection. In the few cases where climax animals could not be obtained and the animals in the culture were not all in autogamy, the percentage autogamy was estimated from a stained sample and isolations were made after this estimation. Reduced viability values were then corrected for the percentage autogamy. In all other experiments either climax animals were selected or the animals in the cultures were all in autogamy and selection was unnecessary.

TABLE 4. Experiment 5

Fraction of clones of reduced viability at autogamy. Subcultures from single treated animals. First through fifth fissions after exposure. Based on samples of 36 autogamous animals.

ANIMAL NUMBER	CULTURE NUMBER						χ^2	d.f.	P
	1	2	3	4	5	6			
1*	.000	.000	.389	.000	.111	.000	56.7	5	$<.01$
2*	.028	.088	.000	.000	.167	lost	14.3	4	$<.01$
3*	.000	.028	.000	.000	.000	died	5.0	4	$.20<P<.30$
4	.511	.083	.083	.250	—	.028	40.6	4	$<.01$
5	.175	.556	.639	.639	.760	.472	31.0	5	$<.01$
6	.194	.222	.556	.472	.216	.389	13.2	5	$.02<P<.05$
7	.389	lost	.864	.639	.370	—	24.2	3	$<.01$
8	.222	lost	.389	.167	.111	—	14.9	3	$<.01$
9	.286	lost	.384	.422	.216	—	35.6	3	$<.01$
10	.611	.583	.694	.694	.762	—	0.9	4	$.90<P<.95$
11	.734	.639	.639	.361	.583	.760	6.0	5	$.30<P<.50$
12	.123	.339	.339	.526	.500	.491	13.1	5	$.02<P<.05$
13	.056	.333	.167	.348	—	.472	25.0	4	$<.01$
14	.806	.611	.877	died	1.00	—	13.1	3	$<.01$

* Controls.

The results of experiment 5 are given in table 4. In brief, the results indicate that significant differences exist between two or more cultures in the progeny of the treated animals except for animal numbers 10 and 11.

In general, the control animals gave low reduced viability although certain cultures indicate heterogeneity of the animals. Cultures 3 and 5 derived from animal number 1 and culture 5 from animal number 2 gave values significantly different from zero (0.95 probability) and are responsible for the low probabilities given in table 4 for these animals. It seems probable that these three

values represent three distinct spontaneous changes because sister cultures failed to show reduced viability values significantly different from zero.

The data obtained from the animals exposed to nitrogen mustard may be interpreted in a manner similar to those of experiment 4, i.e. the results are compatible, in a general way, with the hypothesis of delayed mutations. It should not be surprising, however, if some of the observations are difficult to reconcile with this view because of the many possible changes induced by the action of nitrogen mustard. The following experiment presents data of this nature.

Experiment 6. The procedure was the same as for the preceding experiment except that each clone was allowed to undergo seven fissions before a single animal was isolated and the fission products separated at subsequent fissions. The exposure was for five minutes to 0.468 mg/ml nitrogen mustard. The results of this experiment are shown in table 5.

TABLE 5. Experiment 6

Fraction of clones of reduced viability at autogamy in subcultures from single treated animals. Eighth through the eleventh fissions. Based on samples of 36 autogamous animals.

ANIMAL NUMBER	CULTURE NUMBER					x^2	d.f.	P
	1	2	3	4	5			
1*	.028	.000	.028	.000	.000	3.6	4	.30<P<.50
2*	.000	.028	.028	.000	.000	3.6	4	.30<P<.50
3*	.000	.000	.056	.028	.028	4.2	4	.30<P<.50
4*	.000	.000	.000	.000	.028	3.7	4	.30<P<.50
5*	.000	.000	.000	.000	died	0.0	3	>.99
6	.611	.750	.611	—	.167	28.1	3	<.01
7	.000	.056	.028	.139	.083	7.4	4	.10<P<.20
8	.139	.083	.139	.083	.083	1.3	4	.80<P<.90
9	.000	died	.000	.028	.056	4.1	3	.20<P<.30

* Controls.

Only one of the four animals, number 6, exposed to nitrogen mustard was affected to a sufficient degree to show significant differences between derived clones. Animal number 8 and possibly animal number 7 showed slight effects of the treatment.

The differences between the tenth and eleventh fissions in the cultures derived from animal number 6 are significantly different from one another (0.95 probability). Unfortunately, values for both the eleventh fission products were not obtained. It is difficult to explain the differences obtained from the progeny of this animal on the basis of delayed mutations.

The reduced viability values obtained from the other cultures are not at variance with the hypothesis of delayed mutation. Neither do they support it particularly. To account for the relatively low reduced viability in the other cultures it may be assumed that, within a culture, not all the animals contained lethal mutations.

TABLE 6. Experiment 7

Fraction of clones of reduced viability at autogamy in subcultures from single treated animals. Nineteenth through twenty-third fissions. Based on samples of 36 autogamous animals.

ANIMAL NUMBER	CULTURE NUMBER						x^2	d.f.	P
	1	2	3	4	5	6			
1*	.056	lost	.000	.000	.028	.000	5.9	4	.20<P<.30
2*	.028	.000	.000	.000	.028	.028	3.6	5	.50<P<.70
3*	.028	.083	.028	.000	.028	.000	6.0	5	.30<P<.50
4	.916	.888	.750	.695	.972	.888	15.9	5	<.01
5	.695	.750	lost	.778	.832	.832	1.3	4	.80<P<.90
6	.000	.028	.000	.056	.028	.028	0.4	5	>.99
7	.028	.000	.000	.000	.000	**	4.0	4	.30<P<.50
8	.361	.250	.306	.278	.417	**	3.4	4	.30<P<.50
9	.472	.681	.805	lost	lost	**	8.1	2	.01<P<.02

* Controls.
** Not isolated.

Experiment 7. This was performed in the same manner as the previous experiments already discussed except that the cultures derived from the treated animals were maintained in daily isolation cultures for 18 or 19 fissions before single animals were isolated and their fission products separated at each fission. The animals were exposed for five minutes to 0.506 mg/ml nitrogen mustard and autogamy was induced in the derived cultures as before. Samples of 36 autogamous animals were isolated. The results of carrying out this procedure on the animals whose progeny had undergone 18 fissions before isolation are given in table 6.

Table 7 shows the results obtained from the cultures which had undergone

TABLE 7. Experiment 7

Fraction of clones of reduced viability at autogamy in subcultures from single treated animals. Twentieth through twenty-fourth fissions. Based on samples of 36 autogamous animals.

ANIMAL NUMBER	CULTURE NUMBER						x^2	d.f.	P
	1	2	3	4	5	6			
1*	.167	.111	.000	.028	.000	.000	19.4	5	<.01
2*	.028	.000	.000	.000	.000	**	4.0	4	.30<P<.50
3*	.000	.000	.000	.000	.000	**	0.0	4	>.99
4	.028	.000	.000	.028	.028	.000	3.6	5	.50<P<.70
5	.832	.805	lost	.778	.860	.750	1.9	4	.70<P<.80
6	.000	lost	.028	.000	.000	.056	5.9	4	.20<P<.30
7	.000	.000	.000	.000	.000	.000	0.0	5	>.99
8	.000	lost	.000	.000	.000	.000	0.0	4	>.99
9	.028	.000	.000	.000	.000	.000	2.0	5	.80<P<.90

* Controls.
** Not isolated.

19 fissions before isolation of single animals for investigation. Only 5 of the 12 animals exposed to nitrogen mustard were affected by the treatment. One, possibly two, of the exposed animals from the 18 fission group showed significant heterogeneity (animals numbers 4 and 9) while none of the 19 fission group developed differences. Thus, either most of the cultures had stabilized with regard to mutations or differences had never arisen in the progeny of most of the exposed animals. The latter seems unlikely in view of the number of differences found in experiment 5.

Irrespective of the true nature of the changes involved in the reduction of viability at autogamy, certain conclusions may be drawn from the preceding experiments concerning the general behavior of these changes.

1. Significant differences between cultures derived from the same animal frequently arise.
2. The frequency with which these differences arise decreases with the number of fissions since exposure to nitrogen mustard. Thus, during the 18–24 fissions only 2 such differences were obtained, while during the first five fissions more than a half of the animals gave rise to significant differences.
3. The fraction of progeny of reduced viability may rise or fall in successive fissions.

Most of the differences found between cultures derived from a single treated animal and the decrease in the frequency with which these differences arise when the clones are allowed to undergo a number of fissions are compatible with the hypothesis that exposure to nitrogen mustard produces frequent lethal mutations and unstable loci, resulting in delayed mutations. This is what would be expected if the effects in Paramecium were similar to those in Drosophila.

Breeding experiments are required to discover the nature of the physical basis for the inheritance of reduced viability. The formal explanations presented in the present section are to be accepted only as not in conflict, in general, with the hypothesis derived from consideration of nitrogen mustard effects in other organisms. Other interpretations of these data are possible but, again, would be formal in nature.

The data of the foregoing experiments also fail to distinguish between the various possible types of nuclear effects. Thus, changes in viability due to translocations, other chromosome aberrations or variation in penetrance of gene mutations would not have been identified as such. Neither would effects on the cytoplasm have been identified from these experiments. One of the difficulties encountered in investigating animals exposed to nitrogen mustard is that, to investigate sufficient numbers of treated animals, small samples of their autogamous progeny must be used. Sampling error is correspondingly high and this results in uncertainties as to how many changes of a given type will account for a given value for reduced viability. Furthermore, delayed mutations in a clone, after it has undergone several fissions, will result in values of reduced viability at autogamy which may not fit simple genetic ratios. Future experiments are planned to take some of the difficulties into account. Successful analysis of a specific case will depend upon obtaining relatively rare simple

changes. Unfortunately, much of the analysis must be in process before one knows whether the material is or is not favorable. Attempted analyses of complex cases may give valuable information concerning the behavior of nitrogen mustard effects in general. With such knowledge, experiments may be designed in which simpler changes may possibly be obtained.

D. *Transmission of effects to untreated animals by conjugation*

Experiment 8. A single animal was exposed to the action of nitrogen mustard (0.225 mg/ml) for five minutes and subjected to the following procedure. After several fissions the clone was subdivided into two subcultures, one of which served as a source for autogamous animals, the other serving as a source for animals to be mated to normal animals. Reduced viability at autogamy was found to be 0.460. Fifteen F1 pairs were secured in which the time for separation after conjugation was observed (a measure of cytoplasmic exchange).

TABLE 8. Experiment 8

Reduced viability at autogamy after conjugation. Based on samples of 30 autogamous animals.

PAIR NO.	SEPARATION TIME IN MINUTES (MAXIMUM)	REDUCED VIABILITY AT AUTOGAMY PAIR MEMBER	
		a	b
4	0	.000	.000
3	1.5	.866	.667
10	2	.433	.400
11	2	.000	.000
14	4	.667	.866
9	7	.467	.533
15	7	.500	.600
12	10	.033	.033
5	13	.600	.267
8	13	.500	.400
6	19	.533	.400
7	21	.000	.000

Autogamy was induced in each pair member which had not died and the reduced viability of the F2 generation determined. The results of this procedure are shown in table 8. Three of the pairs, not shown in the table, had one pair member which died. One of the three surviving cultures showed reduced viability at autogamy (0.400) while the other two gave no reduced viability.

Four of the twelve pairs of which both pair members were viable gave insignificant reduced viability at autogamy (F2 generation) in either pair member while six pairs gave approximately 0.50 reduced viability in each pair member. Two pairs gave approximately 0.75 reduced viability in each pair member. In no pair was there a significant difference between the pair members regardless of how short the separation time. Hence, cytoplasmic exchange was not needed to give equal pairs and therefore the basis must be nuclear in this case. Control pairs showed insignificant reduced viability at autogamy.

TABLE 9. Experiment 8

Reduced viability at autogamy following the crosses indicated. Based on samples of 30 autogamous isolations.

F1 CULTURE NUMBER (TABLE 8)	F1 CULTURE MATED TO CULTURE NO.	PAIR NO.	REDUCED VIABILITY AT AUTOGAMY PAIR MEMBER	
			a	b
10b	10a	1	—	.301
		2	.534	.700
		3	.267	.367
10b	normal	1	.000	.000
		2	.000	.067
		3	.367	.667
		4	died	.134
		5	.000	.000
		6	.033	.000
6b	normal	1	died	.367
		2	.467	.667
		3	.400	.301
		4	.434	.400
		5	.534	.033
3a	3b	1	died	died
		2	died	.634
		3	died	died
		4	.935	.534
		5	.967	died
5a	5b	1	.033	.000
		2	1.000	died
		3	died	.634
8a	kb	1	died	.367
		2	died	died

Matings between various F1 cultures were made and are given in table 9. These data indicate that though nuclear inheritance is involved, the situation is complex and cannot be simple gene changes.

Experiment 9. An animal was exposed to 0.213 mg/ml nitrogen mustard for ten minutes. At autogamy the progeny showed 0.66 reduced viability in a sample of 100 isolations. One subculture of the culture derived from the treated animal was crossed to normal animals and the pairs isolated. By allowing the cultures to grow at maximum fission rate selfing was prevented. After each culture had undergone three or four fissions, a single animal was isolated from each culture and allowed to grow to provide cultures for the induction of autogamy and for mating with normal animals. In addition, observations for reduced viability were made on the cultures from which the isolations had been

made. In 30 of 59 (0.509) both pair members were of reduced viability. Since the pair members had not been separated after conjugation it is not known whether there were any pairs in which only one pair member was affected. Crosses to normal animals of the subcultures mentioned were made as soon as the cultures became sexually reactive. The original treated animal was of mating type VII. Presumably, mating type may be used as a marker with little error. Treatment with nitrogen mustard increases the frequency of mating type change at fertilization only slightly (unpublished). Thus, the F1 cultures used in the second cross which were mating type VII were probably derived from the treated member of the pairs. Those of mating type VIII were presumably from the untreated pair member. The F2 obtained in this experiment was obtained from only one mating type of each pair.

Two mating type VII cultures were crossed to normal animals and a total of 47 pairs obtained of which one was of reduced viability. Four mating type VIII cultures were crossed to normal animals and a total of 64 pairs obtained of which 31 were of reduced viability (0.484). In other words, cultures presum-

TABLE 10. Experiment 9

Reduced viability at autogamy after conjugation. Based on samples of 30 autogamous animals.

PAIR NO.	MATING TYPE OF LINE SELECTED FOR INDUCTION OF AUTOGAMY	REDUCED VIABILITY AT AUTOGAMY AFTER FIRST CONJUGATION	REDUCED VIABILITY AT AUTOGAMY AFTER SECOND CONJUGATION
1	7	0.699	0.933 0.800 0.767 0.733 0.667 0.600 0.500 0.667 0.500 0.333
2	7	0.733	0.000 0.800 0.867 1.000
3	8	1.000*	0.833 0.767 0.733 0.733 0.700 0.533 0.500 0.100 0.000
4	8	0.933	1.000 1.000 0.867
5	8	1.000*	0.800 0.767 0.100 0.000
6	8	0.933	
7	?	0.500	
8	?	0.567	
9	?	0.933	
10	?	0.667	
11	?	0.500	
12	?	0.700	
13	?	0.600	
14	?	1.000	
Mean		0.726	0.617

* Clones which are only slightly reduced in viability are capable of conjugation, subsequent pairs giving rise to normal vegetative clones until the next autogamy at which time reduced viability is manifested.

ably derived from the untreated member of the pairs were more non-viable than were cultures derived from the treated member of the pairs when crossed to normal animals. This behavior has been noted in several experiments. All the cultures showed reduced viability at autogamy. Eleven pairs were obtained from the original F1 cultures when they were allowed to become underfed to induce mating. The eleven pairs were from two of the cultures from which mating type VIII isolations had been made. Four of the eleven pairs were of reduced viability after conjugation.

From the foregoing experiments it is evident that the cultures derived from untreated pair members of a cross between treated animals and untreated animals may give more progeny of reduced viability than the cultures derived from the treated members of pairs. The transmission of the physical basis of reduced viability indicates nuclear factors but the fact that the untreated mate may show the effects to a greater degree would indicate that other factors may be involved also.

Of the pairs surviving the first cross in experiment 9 fourteen were successfully investigated further. Autogamy was induced in the F1 cultures derived from the single isolations made and samples of 30 autogamous animals isolated. As mentioned previously the cultures were also mated again to normal animals. From the pairs secured in this mating single isolations were again made and autogamy induced in their progeny. Samples of 30 autogamous animals were isolated and observed for reduced viability. The results of these procedures are shown in table 10. It will be noted that each of the 14 F1 cultures gave progeny of reduced viability at autogamy. The mean reduced viability of the F1 pairs was 0.726 and after the second conjugation the mean reduced viability at autogamy was 0.617. If the effects had been entirely genic, one would have expected that the mean would have been halved.

Experiment 10. Four mating type VII animals were exposed to the action of nitrogen mustard (0.562 mg/ml) for 6.5 minutes, allowed to multiply and mated to normal animals (BC1 generation). These pairs were separated after conjugation in order to detect unlike pair members. Some of these cultures were saved and backcrossed again to normal animals (BC2 generation). Only cultures from pairs in which both members were normal were used in the second cross. Results of these crosses are given in table 11. It will be noted that the reduced viability after conjugation increased in all but two cultures in the second backcross. The fact that the reduced viability effects were transmitted to the untreated member of the pair in every case (except number 4) seems to indicate that nuclear inheritance is involved. The fact of transmission of effect also indicates that conjugation took place in the pairs. Data from number 4 will be considered in a later section (page 271).

In two of the crosses the untreated member of the pair (mating type VIII) gave a higher value for reduced viability than did the cultures derived from the treated member. All the pairs carried reduced viability effects in both pair members, and while the results are not those expected if only simple genic differences existed, they conceivably could result from interference with meiotic behavior of the chromosomes. On this view it is difficult to understand why the

TABLE 11. Experiment 10

Reduced viability after conjugation. Two successive backcrosses to normal animals of cultures carrying reduced viability effects.

SOURCE LINE NO.	CLASSIFICATION OF BC1 PAIRS*			PAIR NO.	MATING TYPE	CLASSIFICATION OF BC2 PAIRS*			REDUCED VIABILITY	RATIO BC2/BC1
	NN	Na	aa			NN	Na	aa		
1	4	10	1	4	8	0	2	14	0.938	2.34
					7	0	0	12	1.000	2.50
	Reduced viability 0.400			12	8	0	3	13	0.906	2.26
					7	9	20	9	0.500	1.25
2	2	9	3	17	8	16	22	1	0.308	0.57
					7	4	8	13	0.680	1.27
	Reduced viability 0.537			30	8	0	9	18	0.833	1.55
					7	3	4	2	0.445	0.83
3	2	7	0**	38	8	0	4	1	0.600	1.54
					7	0	4	21	0.920	2.37
4	6	6	0	40	8	8	0	0	0.000	
					7	0	10	2	0.584	
				41	8	13	0	0	0.000	
					7	0	7	2	0.612	
	Reduced viability 0.250			45	8	17	1	0	0.028	
					7	1	3	2	0.583	
				46	8	17	11	1	0.224	
					7	11	1	0	0.042	

* NN=both pair members normal, Na=one member normal, one member affected, aa=both members affected.

** Reduced viability after conjugation 0.389.

reduced viability increased in the second backcross and was carried in every pair.

E. *General discussion*

The data given in the preceding section may be divided into two groups: 1. Data which are proof that nuclear inheritance is involved and 2. Data which are compatible with such inheritance. In addition there are a few data which cannot be readily explained as due to nuclear inheritance.

The data which give proof that nuclear inheritance is involved were obtained from the experiments in which breeding analysis was carried out. Conclusive proof was obtained in experiment 8 which demonstrated that reduced viability effects could be transmitted, in the absence of cytoplasmic exchange, to non-treated mates. Contributory evidence was obtained in this experiment by intermating some of the F1 cultures and determining the reduced viability at autogamy after conjugation.

In experiment 10 it was possible to obtain greater, or at least no less, reduced viability after conjugation in the untreated mates of several pairs. The

fact that so many pairs showed transmission of the physical basis of reduced viability and the fact that the non-treated member failed to show less reduced viability than the treated member makes it extremely likely that the nucleus was involved in the transmission. Experiment 9 yielded data bearing out this conclusion.

Most of the data obtained from the study of heterogeneous clones are in keeping with what one would expect on the hypothesis that the effects of nitrogen mustard in *Paramecium aurelia* are much the same as the effects in other organisms. The fact that reduced viability is manifested only at fertilization lends credibility to this view.

Thus, there is little doubt that a nuclear basis is involved in many of the reduced viability effects presented so far. The exact nature of the changes in the nuclear material responsible for these effects is not clear.

RESULTS SEEMINGLY INCOMPATIBLE WITH THE ASSUMPTION OF A NUCLEAR BASIS

A. *Transmission of the physical basis through successive autogamies*

If a clone which carries a nitrogen mustard effect is allowed to undergo autogamy and a number of animals isolated, a certain fraction of them will be of reduced viability when observed at the end of the four day growth period. If some of the survivors are allowed to undergo autogamy and are sampled in the same way, sometimes reduced viability is again expressed in some of the autogamous isolations. In other words, the effect manifested as reduced viability at autogamy persists through successive autogamies. In one such experiment, reduced viability was observed for four successive self-fertilizations. Indeed, there is no evidence that the effects cannot be perpetuated indefinitely in this manner.

Experiment 11. Surviving clones from a sample of 50 autogamous animals from an affected clone (0.440 reduced viability at the first autogamy) which had been derived from an animal subjected to 0.169 mg/ml nitrogen mustard for three minutes were allowed to undergo a second autogamy by the tube method and samples of 24 autogamous animals were isolated. Of the eight A1 clones successfully followed, one showed 0.333 reduced viability after the second autogamy. The procedure was repeated on five survivors of this one clone. Two of the five clones showed significant reduced viability at autogamy (0.209 and 0.125). Twelve of the survivors from the clone showing 0.209 reduced viability were allowed to undergo autogamy in mass culture and observations were made periodically. Two of the twelve cultures showed dead and dying animals under these conditions. The experiment was then terminated. A summary of the data obtained in this experiment is presented in table 12. The number of clones maintained during the course of this experiment was too small to demonstrate either an increase or a decrease in degree of effect, if either occurred. This experiment shows that apparently normal animals, from a clone which will show reduced viability at autogamy, may carry reduced viability effects from one fertilization to another even though they are not affected themselves during vegetative reproduction.

One possible explanation of these results might be that of variable penetrance of a detrimental gene. Penetration would be determined at autogamy; any animal not being affected at the time of fertilization would be capable of surviving until the next fertilization at which time the gene would again cause a certain proportion of the individuals to be of reduced viability. This explanation is inadequate, for it would require all clones of the same genotype to have the same fraction of reduced viability under the same conditions. After the first autogamy, all descendants must be alike in genes. Yet the reduced viability was confined to a small proportion of the A2, 3 and 4 sub-clones. Cultures

TABLE 12. Experiment 11

Transmission of physical basis of reduced viability through successive self-fertilizations.

FIRST AUTOGAMY	REDUCED VIABILITY AT AUTOGAMY					
	CULTURE NO.	2ND AUTOGAMY	CULTURE NO.	3RD AUTOGAMY	CULTURE NO.	4TH AUTOGAMY
0.440	1	0.333	1	0.209	1	Yes
	2	0.042	2	0.125	2	Yes
	3–8	0.000	3–5	0.000	3–12	No

1–8 derived from the survivors of the first autogamy could have been of varying genotypes and thus give different results at the second autogamy. However, the cultures surviving the second autogamy, derived from the culture 1 (0.333 reduced viability) must necessarily have possessed the same genotype and therefore have given identical results.

The occurrence of several clones which gave no reduced viability at autogamy in these generations is sufficient to eliminate the possibility of variable penetrance as an explanation in this case. Delayed effects of the nitrogen mustard fail to provide an adequate explanation, also, in view of work which indicated little delayed effect after 18–24 fissions (experiment 7). By the fourth autogamy there have been 60–70 fissions since exposure to nitrogen mustard.

Another possible explanation is based on cytoplasmic factors. One may postulate cytoplasmic factors having effects only at the time of fertilization; at other times not being capable of expression. The concentration of such factors would be important in determining the degree of reduced viability at autogamy.

B. *Percentage reduced viability not decreased in successive autogamies and outcrosses*

Experiment 10 (see also page 268). As indicated in a previous section the reduced viability increased at the second backcross to normal animals. Nuclear inheritance seemed to be involved in three of the four cases reported but the fourth case cannot be reconciled with nuclear inheritance. The data obtained from crossing source line number 4 (table 11) are in keeping with what would be expected if cytoplasmic inheritance were involved. Backcrossing the cultures obtained from pairs 40, 41 and 45 indicated that only the cultures of

mating type VII gave reduced viability after conjugation. A limited number of animals from these cultures gave pairs in which both pair members were of reduced viability but these might be expected if cytoplasmic exchange had occurred in these pairs. Pair number 46 showed reduced viability to a significant degree only in the culture of mating type VIII which was presumably derived from the untreated member of the pair. This is exceptional but may be explained if both pair members had changed mating type during conjugation. In view of the lack of mating type change in the other pairs this seems very unlikely.

In addition to the exceptional data obtained from source line number 4 in this experiment certain of the results obtained from the other source lines seem to suggest that more than nuclear inheritance is involved. It will be noted from table 11 that every pair investigated carried reduced viability effects. To show the relative reduced viability between the first and second backcross the ratio of the reduced viability at the second backcross to that of the first backcross may be calculated. It will be noted that in the cultures from source line 1 the reduced viability at the second backcross was more than doubled. The reduced viability in the cultures from source line 2 remained approximately the same while in the cultures from source line 3 the reduced viability increased. In the cultures which carried the effects from source line 4 the reduced viability was approximately doubled. However, in half the cultures reduced viability failed to appear. Thus, if an average is calculated, the effects remained at approximately the same level in this case.

The fact that the degree of reduced viability failed to decrease, actually increasing in several crosses, is not expected if only nuclear inheritance is involved. It seems probable, therefore, that extra-nuclear inheritance is indicated.

Experiment 8 (see also page 265). The mean reduced viability of all cultures shown in table 8 in the F2 generation was 0.543 while the reduced viability at autogamy of the clone derived from the treated animal was 0.460, indicating that the degree of reduced viability at autogamy was not decreased by conjugation. One conjugation interposed between determinations of reduced viability at autogamy should, if genes are responsible for reduced viability, result in a halving of the degree of reduced viability. Since this does not happen, something other than genic inheritance must enter into the inheritance. While chromosomal aberrations cannot be excluded by the data presented, no known chromosomal configuration seems adequate to explain them.

Experiment 9 (see also page 266). The mean reduced viability at autogamy after the first conjugation was 0.726 (table 10) as opposed to the value of 0.660 obtained at the first autogamy in the culture derived from the treated animal. After the second conjugation the mean reduced viability was 0.617. None of these values is significantly different from the others. The decrease in degree of reduced viability at autogamy, required by the nuclear hypothesis, did not occur. In addition, as pointed out on page 268, the cultures of different mating types behave in different ways. This does not seem to be explicable on a nu-

clear basis. The failure to halve the degree of reduced viability by means of conjugation has been verified in other experiments.

C. *Discrepancies from expected equivalence of mates*

Experiment 12. A single animal exposed to nitrogen mustard for three minutes (0.158 mg/ml) was allowed to undergo one fission, one product being taken to establish a culture in which autogamy was induced and a sample of 24 animals isolated. Three of the 24 clones derived from these isolations were of reduced viability (0.125). The other first fission product was allowed to multiply at maximum fission rate and the resulting clone was mated to normal animals, the separation time for the pairs being observed. Four pairs were secured in which the separation time was zero and one pair in which it was two minutes. This means that cytoplasmic exchange was at a minimum in these pairs. Each pair member was individually isolated after conjugation and autogamy was induced in each culture as rapidly as possible by the tube method. Samples of 24 autogamous animals were obtained and observed for reduced viability. The results are shown in table 13. The pair members were unlike as regards reduced viability at autogamy. This is suggestive of cytoplasmic inheritance. Conclusive proof that the pairs actually mated cannot be offered. However, the results of experiment 10 (page 268) seem to indicate that mating occurs regularly in material such as this.

TABLE 13. Experiments 12–13

Reduced viability at autogamy after conjugation based on samples of 24 autogamous animals.

EXPT.	PAIR NO.	SEPARATION TIME IN MINUTES (MAXIMUM)	REDUCED VIABILITY AT AUTOGAMY PAIR MEMBER	
			a	b
12	1	0	0.584	0.000
	2	0	0.835	0.000
	3	0	0.460	0.000
	4	0	0.000	0.333
	5	2	0.292	0.000
13	1	0	0.000	0.333
	2	32	0.460	died
Controls	1	0	0.000	0.000
	2	22	0.000	0.000

Experiment 13. A single animal, treated as in the preceding experiment, was found to give 0.333 reduced viability at autogamy. Two pairs were obtained in a mating to normal animals: one pair had zero separation time and the other had a separation time of approximately 30 minutes. The first pair showed that the pair members were different while in the second pair one member died in vegetative reproduction and the other carried the effect as shown by isolations

at autogamy (table 13). The fact that one pair member died is not to be considered unusual if the results of experiment 10 (page 268) are taken into account. These results indicate that this mortality may be of common occurrence. While the results of experiments 12 and 13 make cytoplasmic inheritance the most likely explanation, they are not conclusive. To obtain conclusive proof it would be necessary to investigate an effect the transmission of which can be correlated with cytoplasmic exchange in several conjugant pairs. This was impractical in the present work for various technical reasons.

Experiment 8. The cultures used in this part of the experiment have been discussed on pages 265 and 272, as have been the procedures used in obtaining them. Certain of the F1 cultures obtained were mated in all combinations, the pair members being isolated individually after conjugation, and the resulting clones observed for reduced viability. The results are shown in table 14. The number of pairs in the N-a groups (one member normal and one of reduced viability) is too large to explain on a nuclear basis and too small to explain entirely on a cytoplasmic basis.

TABLE 14. Experiment 8

Results of intermating certain F1 cultures. Reduced viability after conjugation of cultures numbered as in table 8. Fractions are values for pairs of class aa after conjugation.

CULTURE	3a			5a			6b			8b			10b			14a		
	NN	Na	aa	NN	Na	aa	NN	Na	aa	NN	Na	aa	NN	Na	aa	NN	Na	aa
3b	10	16	49	2	17	64	11	18	49	0	32	59	6	20	45	0	6	52
			.653			.770			.628			.649			.633			.898
5b				5	16	17	28	14	13	17	17	14	24	11	17	13	15	30
						.448			.236			.292			.327			.518
6a							29	10	20	102	9	29	24	21	14	5	18	35
									.338			.207			.238			.603
8a										34	8	20	19	48	35	1	20	55
												.322			.343			.725
10a													11	32	18	8	19	62
															.295			.696
14b																0	1	67
																		.985

The differences cannot reasonably be ascribed to delayed mutations because of the age of the cultures which were used in these crosses. As noted in the section on heterogeneous clones, cultures derived from isolations after 18 or more fissions were relatively homogeneous, which fact was taken to indicate a lack of delayed effect after that number of fissions. The crosses in the present experiment were made between F1 cultures from a cross of a culture derived from a treated animal with normal animals. Thus, the cultures would have gone through sufficient fissions for them to have become relatively stable (approximately 20–25 fissions).

D. *Discussion*

The results which are not readily reconciled with the assumption that the changes induced by nitrogen mustard are all nuclear may be summarized as follows. 1. The physical basis of reduced viability is sometimes transmitted

through successive autogamies and cannot be adequately explained on the basis of incomplete penetrance of a mutated gene. 2. The percentage of reduced viability, after conjugation and after autogamy, is not decreased by successive conjugations. Indeed, it is frequently increased. 3. There are serious discrepancies from the expected equivalence of clones derived from the two members of a pair with respect to their survival and the viability of their progeny.

In addition to the data given in the present section, the preceding section also contains results difficult to explain on a nuclear basis. Primarily, the results obtained from animal number 6 (table 5) are difficult to explain. In addition, it is not necessary to explain all the results of the preceding section on a nuclear basis. Some of the data, particularly some of those obtained in the studies on heterogeneous clones, may also be accounted for on extra-nuclear grounds. One might expect cytoplasmic effects to be manifested at fertilization also. This would explain reduced viability at autogamy and variation in degree of reduced viability between sister clones could then be explained on the basis of variation in concentration of a cytoplasmic factor concerned with viability.

DISCUSSION

The expectations of the present study at the outset were twofold. It was thought that the experiments to be performed would result in confirmation of the mutagenic activity of nitrogen mustard on nuclear genetic material and, more importantly, might result in evidence for the mutability of cytoplasmic genetic factors which are known to exist for some traits and are suspected in others in *Paramecium aurelia*. That the data obtained would be so complex and that the effects of the mutagen would be so variable was not anticipated. In spite of these circumstances, certain information has been obtained concerning the changes induced in the heredity of this organism as a result of exposure to nitrogen mustard. Some of the results obtained in the present investigation agree with those found in the study of mutagenesis in other organisms. The data indicate that nitrogen mustard induces hereditary changes in *Paramecium aurelia*. That nuclear inheritance can be involved has been conclusively demonstrated by showing that the physical basis of reduced viability may be transmitted through conjugation irrespective of cytoplasmic exchange. Supporting evidence for nuclear inheritance is gained from the fact that reduced viability is manifested at nuclear reorganization. In addition, the occurrence of genetically heterogeneous clones is in keeping with the fact that one of the prime effects of nitrogen mustard is the production of unstable genes and chromosomes (AUERBACH, ROBSON and CARR 1947). While the data indicate that nuclear factors are involved, they do not reveal the type of nuclear change responsible for the appearance of reduced viability after fertilization, i.e. whether it be chromosomal, genic or gross cytological change. The first of the aims of this study, that of confirmation of the mutagenic activity of nitrogen mustard on nuclear material, thus has been realized though it has not been possible to demonstrate a simple situation which could be easily analyzed.

The segregation of the physical basis of reduced viability through vegetative reproduction may be formally explained on the basis of delayed gene mu-

tation but it could be explained, in part at least, by assuming certain combinations of other nuclear phenomena. Explanations based on such assumptions are less convincing than the simpler hypothesis of delayed mutation. Further investigation with refined techniques should reveal whether any of these other factors enter into the inheritance of reduced viability through nuclear material.

Data from crosses between clones carrying reduced viability effects and normal animals indicate that in some cases members of a pair, theoretically genically identical, differ in the degree of reduced viability that their progeny will show at autogamy and at conjugation. In some of these cases the differences between the members of a pair is correlated with a lack of cytoplasmic exchange; similarity of pair members is coincident with cytoplasmic exchange. In other experiments in which nuclear inheritance is involved there are also differences in behavior between pair members. The occurrence of such differences is too frequent to ascribe to any well known nuclear phenomena. In addition, it has been shown that the reduced viability effects may persist from one autogamy to another which would not be expected if the effects were entirely nuclear. Other data make improbable the hypothesis that nuclear changes alone are responsible for the observed changes. Repeated backcrosses to normal animals fail to decrease the degree of reduced viability manifested at autogamy. These data, plus the fact that in many crosses all the cultures obtained carried the effects, are strongly indicative that some of the mutagenic effects of nitrogen mustard are on the cytoplasm and that cytoplasmic inheritance of reduced viability is possible.

The exact nature of the changes induced in the cytoplasm by the action of nitrogen mustard should be thoroughly investigated by more precise methods in which an attempt at experimental control of the physical basis of such inheritance is made. Attempts should be made to secure additional material in which only gene or chromosomal changes can be demonstrated as well as material in which only cytoplasmic changes have taken place. If separation of the two general types of changes can be obtained, analysis would be much simplified. The subject of the interaction of gene and cytoplasmic factors involved in viability of the organism could then form the basis for many valuable investigations.

SUMMARY

Investigation of changes in viability induced by exposure of *Paramecium aurelia* to nitrogen mustard has resulted in the confirmation of the fact that nitrogen mustard is a potent mutagen.

The evidence presented indicates that genetic changes induced by this compound frequently have a nuclear basis, although the types of nuclear changes involved have not been identified.

Considerable evidence has been presented which suggests that the cytoplasm may also be involved in the inheritance of reduced viability in *Paramecium aurelia*.

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LITERATURE CITED

AUERBACH, C., 1949a Chemical induction of mutations. Proc. 8th Int. Congr. Genetics Hereditas, Suppl. Vol. 128–147.

1949b Chemical mutagenesis. Biol. Rev. **24**: 355–391.

AUERBACH, C., and J. M. ROBSON, 1944 Production of mutations by allyl isothiocyanate. Nature **154**: 81.

1946 Chemical production of mutations. Nature **157**: 302.

1947a The production of mutations by chemical substances. Proc. roy. Soc. Edinb. **62**: B: 271–283.

1947b Tests of chemical substances for mutagenic action. Proc. roy. Soc. Edinb. **62** B: 284–291.

AUERBACH, C., J. M. ROBSON, and J. G. CARR, 1947 The chemical production of mutations. Science **105**: 243–247.

DARLINGTON, D. D., and P. C. KOLLER, 1947 The chemical breakage of chromosomes. Heredity **1**: 187–221.

GECKLER, R. P., 1949 Nitrogen mustard inactivation of the cytoplasmic factor, kappa, in *Paramecium aurelia*, variety 4. Science **110**: 89–90.

KIMBALL, R. F., 1949 The induction of mutations in *Paramecium aurelia* by beta radiation. Genetics **34**: 210–222.

SNEDECOR, G. W., 1946 Statistical Methods. 4th ed. xvi+485 pp. The Iowa State College Press.

SONNEBORN, T. M., 1947 Recent advances in the genetics of Paramecium and Euplotes. Adv. in Genetics **1**: 263–358.

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Kelly, R. B. Principles and methods of animal breeding. Second edition revised and enlarged. New York, John Wiley & Sons, Inc., 1949, viii, 383p. 64 fig. O. $3.50.

Jepson, Glenn L., Mayr, Ernst, and Simpson, George Gaylord, eds. Genetics, paleontology, and evolution. Princeton, Princeton University Press, 1949. xvi, 474p. illus., maps. O. $6.00.

Cleveland, L. R. The whole life cycle of chromosomes and their coiling systems. Transactions of the American Philosophical Society New Series Volume 39, Part I. Philadelphia, June 1949. 100 pages including 35 plates. O. $1.50.

Taylor, L. W. Fertility and Hatchability of Chicken and Turkey Eggs. John Wiley & Sons, Inc., 440 Fourth Avenue, New York 16, N.Y.

Cavan, Burgess, Havighurst and Goldhamer. Personal Adjustment in Old Age. X, 204 p., 1949, Chicago Science Research Associates.

Boletín del Instituto Nacional De Investigaciones Agronómicas, June, 1948. Ministerio de Agricultura, Direction General de Agricultura, Spain.

The Journal of Sex Education. Vol. I, No. I, August, 1948. Published from 127, Harley Street, London, W.I., England. Annual Subscription 10s. 6d in England, 12s. 6 d abroad.

Dürr, Karl. Blutgruppenauswertung für Vaterschaftsprozess, Kriminalistik und Rassenforschung. Verlag Wirtschaft und Recht Bern (Schweiz) 1947. 72p. 1 table.

Physiologia Comparata et Oecologia, An International Journal of Comparative Physiology and Ecology. Vol. I, No. I, 1948. Uitgeverij Dr. W. Junk, Den Haag. 62 p.

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CONTENTS OF PRECEDING ISSUES

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BARRATT, R. W., and L. GARNJOBST, Genetics of a colonial microconidiating mutant strain of *Neurospora crassa*.

MERRELL, D. J., Selective mating in *Drosophila melanogaster*.

HAYS, F. A., and D. W. TALMADGE, Inbreeding in a closed flock.

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KAUFMANN, B. P., and K. WILSON, Modification of the frequency of chromosomal rearrangements induced by X-rays in Drosophila. IV. Posttreatment with near infrared radiation.

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STRØMNÆS, Ø., The production of dominant lethals with X-rays in aged *Drosophila melanogaster* sperm.

SEPTEMBER, 1949

NEWCOMBE, HOWARD B., AND GRACE WORKMAN SCOTT, Factors responsible for the delayed appearance of radiation-induced mutants in *Escherichia coli*

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NOVEMBER, 1949

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JANUARY, 1950

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